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METHODS

OF

PRACTICAL HYGIENE.

METHODS

OF

PRACTICAL HYGIENE

BY

PROFESSOR K. B. LEHMANN
WÜRZBURG

TRANSLATED BY

W. CROOKES, F.R.S.

WITH NUMEROUS ILLUSTRATIONS

IN TWO VOLUMES

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EDITOR'S PREFACE.

In submitting this version of Professor K. B. Lehmann's work on the Methodics of Hygiene, I wish to point out that it is founded not simply on the original German edition of 1890, but on a copy revised, corrected, and extended by the author in accordance with the most recent observations. The translation is literal as far as the character of the languages allows. Where the difference of local circumstances rendered it necessary, footnotes have been added, without any interference with the text. Slight as is the difference between the respective climates of Germany and Britain, the character of the domestic architecture of the two countries, their heating and ventilation, their preferences in diet, &c., not to speak of their sanitary laws, had to be taken into account. The reader will see that in hygienic regulations Germany is in advance of us in some respects, whilst in others she remains in the background. Like ourselves, the Germans are now a manufacturing people, and in consequence they encounter some of the same difficulties which fall to our lot in dealing with unwholesome arts. The adulteration of food is now an established evil in their midst, and it is interesting and instructive to see how they propose to meet it. They are less sensitive than ourselves on the question of sewage-gas, and to the present writer they seem not sufficiently scrupulous as regards the disposal of the dead. Perhaps we may say that, as far as sanitary enactments are concerned, their lawmakers are somewhat less industrious than ours in providing loopholes of escape for offenders; hence a work like the

present may prove useful not merely to our medical practitioners but to our legal experts. On the other hand, quarantines and the isolation of persons suffering from infectious diseases are not discussed.

It will be observed throughout that Professor Lehmann, as a sanitary reformer, does not recommend extreme measures, and rejects unfounded and alarmist assertions.

WILLIAM CROOKES.

December 1892.

PREFACE TO THE ENGLISH EDITION.

I EXPERIENCED great pleasure when Professor Crookes informed me that he was desirous of executing an English version of my book. I have consequently spared no trouble in carefully revising the text, taking into consideration the most recent advances of science up to the latest date, and, as far as lay in my power, supplying the deficiencies which I had previously detected. The bacteriological chapters have undergone considerable remodelling in small details, in accordance with the most recent investigations; many additions have been made to the sections on Dwellings and Disinfection. No portion has been left entirely unmodified. I have sought to avoid the introduction of too many supplemental details, since the purpose of such a work requires exposition of the principles of research and recognition rather than the accumulation of endless details. Even as it is, a sufficient abundance has been introduced. A strict objectivity in the conflict of the opinions of different schools has also been prominently before my mind.

I know full well that, even in the present improved form, numerous questions have remained untouched, or have only been noticed in passing, although they may occasionally concern the sanitarian. Absolute completeness can scarcely be combined with a modest bulk.

As the work has been written by a German, with especial utilisation of the literature available in Germany, and is adapted to German requirements, the necessity for supplemental additions and notes has made itself felt. I hope that

Professor Crookes and his collaborators have succeeded in finding all that is necessary in this direction for English requirements.

And thus I hand over my book to the nation which has taken the lead of all modern civilised peoples in the sphere of practical hygiene. May it experience the same friendly and indulgent reception which has been awarded to it in my own country.

KARL BERNHARD LEHMANN.

WÜRZBURG, *November* 1891.

PREFACE.

In the present work I have had in view two objects. Firstly, I wished to furnish the beginner in the field of hygienic research with a full guide to his investigations, drawn up as intelligibly as possible, but, at the same time, strictly scientific. Above all, the requirements of the physician have been kept in view, both in his private practice, and subsequently as medical officer of health. But not less chemists, apothecaries, municipal officials, and lawyers, as well as teachers of the natural sciences, will find at least certain portions of these instructions useful. For the sake of intelligibility, a minimum of previous acquaintance with chemistry has been assumed.

In the selection of methods, the following principles have been observed: Only well-tried procedures (as far as possible tested by the author himself) have been introduced, such as can be executed without unusual appliances and without the training of the specialist. Only when two equally good methods have conjointly acquired acceptance have both been given; in other cases merely the one in more general use. More difficult procedures, which strictly go beyond the scope of this work, have been appended in small type if it seemed to the advantage of the reader, but for the most part merely in broad outline, with reference to special treatises. The detailed description of large pieces of apparatus, the correct use of which require accompanying printed descriptions, has also been avoided.

Secondly, I have attempted a critical elaboration of the accessible materials for a hygienic decision upon the objects in question. Where it did not seem to me to justify a defini-

tive judgment, I have admitted this openly, even in cases in which the lay public is accustomed to express a decided opinion. It seemed to me more serviceable to point out uncertainties in our knowledge, and thus perhaps contribute to their removal, than to adhere without proof to opinions widely current.

In every decision I have adhered to the principle that the physician and the hygienist are, above all things, concerned with the question whether the object in question is calculated (directly or indirectly) to injure health or not.[1] Only in a secondary respect can the physician entertain the question, whether a manufacturer, producer, or dealer has committed a criminal offence by illicit manipulations (*e.g.*, the use of substitutes of inferior value). The decision in such cases must be left to the judges or to the technical experts called in by the courts. But in the weighty question of an injury to health, every physician should be able to give an independent and well-grounded opinion. So far as I am aware, there is scarcely a modern work in which the medical-hygienic point of view in decisions of this kind is placed in the foreground in the manner here attempted. If I have succeeded in solving the problem, I think that in these sections I have especially supplied the physician with ways and means for a correct hygienic decision, whether he carries out the investigation personally or depends upon the results of a chemist. I hope that chemists also may find these expositions not without interest, as they often deviate widely from the current point of view. Above all, I hope that the present work may be useful in legal circles in throwing light on the requirements of hygiene in so many cases where the letter of the law does not extend, or where experts bring forward conflicting opinions.

[1] According to the ruling of the American judge M'Carter, anything which occasions disgust or annoyance to the public may be suppressed as a nuisance, even if not demonstrably injurious to health.—*Editor.*

The excellent "Text-book of the Methods of Hygienic Investigations" (*Lehrbuch der Hygienischen Untersuchungs Methoden*), by C. Flügge, Leipzig, 1881, has naturally served me in many respects as a model in the plan of my work; but my endeavour, above all things, to supply a work for practical requirements involved very considerable differences in its execution. The mighty advances which hygienic research has made in the last ten years, especially as regards micro-organisms and the analysis of foods, have not been without influence upon the selection of matter, and especially upon the questions raised. During the preparation of the work several handbooks of hygienic investigation have appeared; but as they are principally destined for elementary instruction in hygienic method, and as they lay no emphasis upon medical hygienic decisions, their tendency is essentially different from that of the work before us.

I have everywhere attempted, by means of bibliographic references, to give the reader the opportunity of penetrating more deeply into the subject. Wherever possible, there have been mentioned in the first place recent extended manuals in which the special subject is fully discussed. In citing articles in periodicals, limitation seemed necessary, as approximate completeness could not be aimed at. The literature of the subject has been noticed in the first half of the book about up to November 1889, but in the remaining sheets, as far as practicable, up to June 1890. In a short appendix, I have added the most important matter which has appeared since the printing of the book began, or which I had previously overlooked.

Concerning the standards for water, milk, &c., &c., food chemists are agreed; these figures have not a universal but merely a certain local validity. Each analyst must either ascertain them for his own district or deduce them from the experience of his predecessors. In the opinion of many, general standards are quite worthless. Still it seemed to me

necessary to give the beginner some figures for guidance. It will, *e.g.*, always be better to know that 10 to 35 *mgrm.* of nitric acid are considered the maximum permissible in a litre of drinking-water, than, without any rule, to be forced to decide whether the 50 *mgrm.* found represent a high or a low value. Of course it is always pointed out in what sense such figures have to be accepted.

Gaps and defects will have their explanation, on the one hand, in the naturally defective character of our hygienic knowledge, and on the other—remembering the limits of extent prescribed by purely practical considerations—in the great difficulty of doing uniform justice to all parts of so mighty a subject. A decision is further rendered difficult by the constant development which our hygienic knowledge is experiencing day by day, and lastly by the incompleteness of the available literature. May I ask the reader kindly to take these difficulties into consideration.

Finally I must express my warmest thanks to my friend Dr. F. J. Herz, at present Director of the Memmingen Station for the Examination of the Production of Milk and the Technics of Brewing, for the unwearied care with which he revised the portion of my MS. referring to articles of food, and for the many improvements due to his advice. For Section XVII. I received much practical advice and a number of practical additions from my friend Ch. Nussbaum, architect, of Hanover. I am happy to take this opportunity of publicly thanking him for this assistance.

KARL BERNHARD LEHMANN.

WÜRZBURG, *July* 1890.

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PART I.

GENERAL METHODS.

THE execution of every individual hygienic investigation presupposes an acquaintance with the principles of certain physical, chemical, and biological methods which, to avoid repetitions, are preferably premised in the form of a general part. The study of this part will be especially useful for those who do not enjoy personal instruction on the part of a teacher, though it will also be useful for reference to those who have acquired the principles of research in their university curriculum.

SECTION I.

CHEMICO-PHYSICAL METHODS.

1. The Most Important Chemical Laboratory Work.

§ 1. **Evaporation and Drying.**—If a watery solution has to be evaporated to dryness it is placed in a suitable porcelain capsule on a wire gauze, or a sheet of compressed asbestos, over a non-luminous gas flame. On the wire the liquid boils rapidly, but slowly on an asbestos sheet. If placed over the naked flame even good porcelain capsules readily break if they are heated when full of a cold liquid; the use of a mushroom burner (or a furnace with a number of minute flames in place of one large burner) obviates this danger, and allows of the regulation of the evaporation.

In case of the evaporation of highly concentrated liquids which readily spirt, or such as contain alcohol or other organic

matter, readily coagulable or decomposable, and generally in quantitative operations, the evaporation is to be effected on a water-bath.

The porcelain capsule *c* rests in a metal vessel partially filled with water, so that the contents of the capsule are heated merely by the ascending steam.

To avoid the necessity of frequently replenishing the water-bath we use either a water-bath containing but little water (*a*, Fig. 1, Victor Meyer's design), in which the steam is condensed in the tube *b* and flows back again, so that

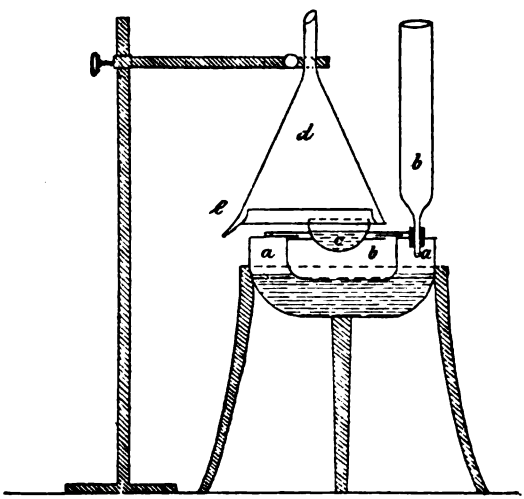


FIG. 1.—Victor Meyer's Water-Bath.

after being used for hours but few cubic centimetres of water escape, or we employ a constant water-bath (Fig. 2).

Here a very slender stream of water is admitted by the tube *a*, thus maintaining in the water-bath a uniform level, *d*, without reducing its temperature by the trifling influx and efflux of water at *b*.

In order to prevent dust from falling into the capsule an inverted glass funnel, *d*, is placed over it, preferably one where the margin is turned up within, leaving an exit for water (*e*, Fig. 1), as proposed by Victor Meyer.

For evaporating large quantities of liquid they are generally first concentrated on the wire gauze, evaporated to dryness on the water-bath, and the desiccation is then completed in the drying-cupboard (a box of sheet-copper with perforated shelves).

For substances not very sensitive to heat a simple box is sufficient, whilst for such as are readily decomposed the temperature must be capable of regulation. A water-jacket or air-jacket gives a good protection against the great

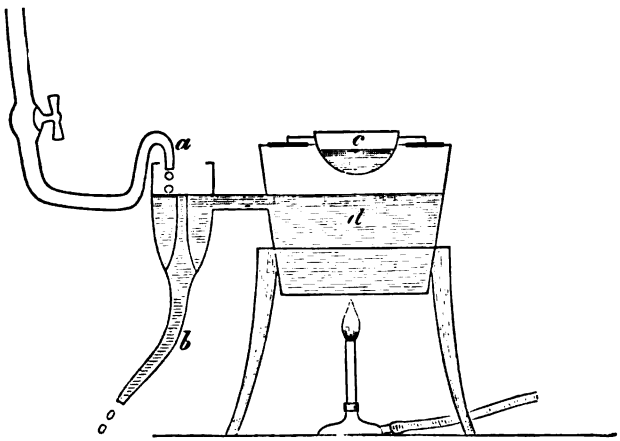


FIG. 2.—Constant Water-Bath.

changes of temperature, but for exact experiments the introduction of a heat-regulator is necessary, which is generally set at 100°. (For particulars see § 62.)

The drying-closets serve also for drying filters, for determining moisture in solids, &c.

§ 2. Mention must here be made of the construction and the right use of the *Bunsen burner* commonly employed in laboratories.

At *o* (Fig. 3) air enters the tube of the burner, drawn in by the hot ascending gaseous current of the flame, mixes with the gas issuing from the fine triangular aperture *n*, thus effecting the complete combustion of the latter; the flame is blueish, non-luminous, but very hot. If the air-entrances *o*

are closed by turning a ring, the supply of air ceases, the flame becomes yellow, luminous, smoky, and produces little heat.

For chemical operations a non-luminous flame is exclusively used, hence cheap burners are often made without the closing-ring.

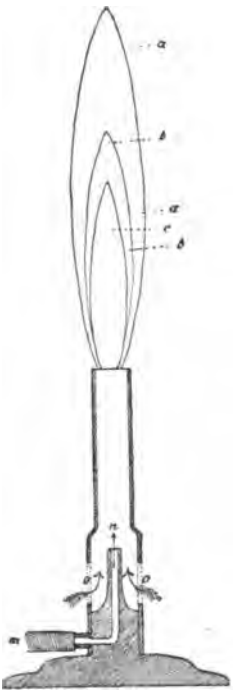


FIG. 3.—Bunsen burner.

The greatest heat is found at the pale violet point of the flame and at its margin. The capsule (especially platinum vessels) is not to be introduced beyond the upper third part, *a*. The colourless zone *b*, which surrounds the greenish-blue, cool, internal core of the flame, exerts a reductive action in consequence of the hydrogen which it contains; the point and the outer surface are the seat of energetic processes of oxidation.

If we wish to work with a small heating-flame, this is best effected by decreasing the current of gas, and at the same time reducing the supply of air by partially pushing forward the closing ring. If this precaution is neglected the flame is apt to strike back, *i.e.*, the gas burns no longer at the aperture of the burner but at the fine slit *n*. There escape then, along with carbon-monoxide, gases of an unpleasant odour, especially acetylene; the burner quickly becomes hot, and is coated internally with soot, whilst the flexible gas-pipe may take fire. As soon as a burner strikes back the flame must be put out and be lighted again. The precaution must first be taken of letting a strong current of gas rush through, kindling it at the burner, and then regulating the flame. A small piece of fine wire gauze fixed over the mouth of the burner is a good precaution against striking back.

In default of gas it is possible to work satisfactorily with

spirit-flames. As a small and temporary source of heat the well-known glass spirit-lamp may be used; for prolonged work and higher temperature we may employ the old and well-tried Berzelius lamp, with an annular brass receptacle for the spirit. For longer operations there may be attached a reservoir for effecting an automatic supply of spirit (Fig. 4).

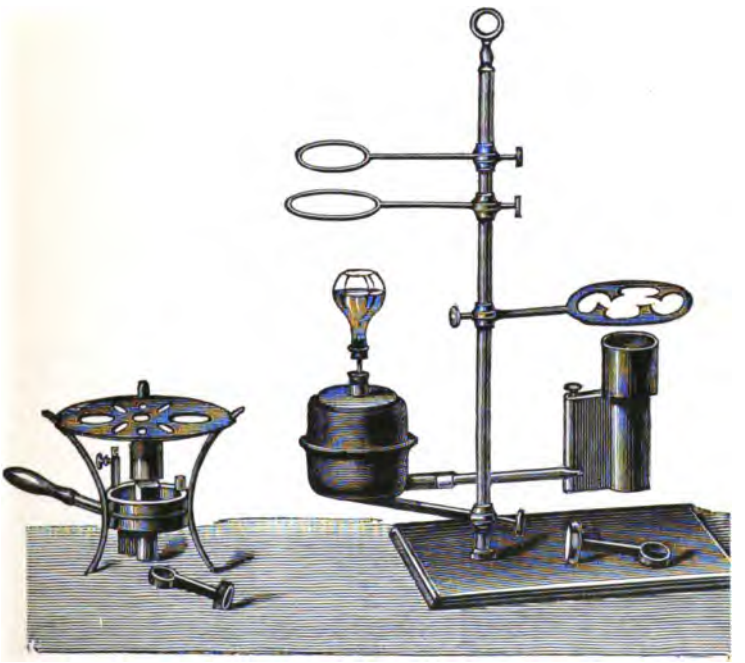


FIG. 4.—On the left an ordinary Berzelius lamp; on the right an improved design, with an arrangement for keeping the level of the spirit constant.

As a substitute for gas there has been recently recommended Barthel's spirit-burner, which takes the place of the Bunsen, and Barthel's benzene-burner, which replaces the gas-blast lamp. The former costs 10s., and the latter 15s.; they may be obtained from all dealers in laboratory requirements. Compare *Chemiker Zeitung*, 1890, No. 14, and 1891, No. 80.

§ 3. **Distillation.**—Volatile bodies may be completely separated from the non-volatile by distillation, and readily volatile liquids may be, to a very considerable extent, removed from such as are not easily volatile. If we place (Fig. 5) in the flask (or the retort) *a* a mixture of bodies differing in volatility (*e.g.*, water and alcohol), on heating the mixture the more volatile liquid—alcohol—escapes first, condenses in the refrigerator *b*, which is surrounded by water, and is collected in the receiver *f*. If the boiling-points of the two bodies lie near together, the distillate naturally contains a portion of the less readily volatile. A complete separation can be effected only by means of special expedients. A cur-

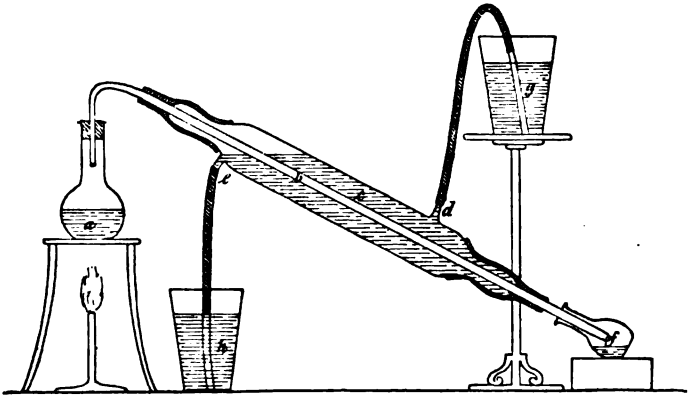


FIG. 5.—Distillation.

rent of cold water from a cistern or water-main, *g*, enters the refrigerating tube at *d*, whilst the heated water escapes at *e*.

In order to avoid bumping (violent, succussive boiling) of the liquid which is being distilled, a spiral of platinum wire is placed in the retort (in the distillation of ether, alcohol, &c.), or fragments of pumice are added. See section: Beer, Wine, Butter.

Reflex-condensers, *see* § 217.

Distillation in a current of steam, *see* Wine. See § 371.

§ 4. **Incineration and Ignition.**—If it is required to isolate the inorganic constituents of a substance, the organic

matter is burnt with the precautions mentioned in the determination of the ash. See § 210.

Such determinations are preferably effected in platinum vessels, whereby the following precautions must be observed: The crucible must be supported only on a triangle of platinum, or one of iron encased in pipe-stems or sheet-platinum, never on copper, brass, or iron.[1] No substance containing copper, lead, silver, gold, tin, iodine, bromine, or caustic alkali must be ignited in the crucible, since otherwise the platinum combines with these substances and the crucible is quickly destroyed.

The crucible must always be heated with the oxidising flame (§ 2), *i.e.*, the point of the flame: in the reducing flame carbon may easily combine with the platinum, and, if it is afterwards exposed to the oxidising flame, the carbon burns away, and the platinum remains in a spongy condition.

For the ignition of precipitates no general rules can be given. In some cases the strongest heat is required with the aid of the blast, *e.g.*, in order to convert calcium carbonate or oxalate into caustic lime; in other cases an intense heat would falsify the results, *e.g.*, by the volatilisation of alkaline salts, &c. In using the gas-blast, a moderately hot flame is first produced by opening the gas-cock widely and opening the air-cock but slightly, or forcing in but little air. Gradually, when the vessel to be heated has become warm (platinum requires no preliminary warming), the current of air is increased, the supply of gas is restricted, and the operation is continued with a small, blue, intensely hot flame. The large Terquem burner which is now met with in commerce quickly fuses a thick copper wire, and enables the experimentalist in small laboratories to dispense with a blast.

§ 5. **Pulverising.**—If a substance is to be pulverised it is dried in the desiccating-box (if not already dry), and ground down either in a porcelain mortar (soft and brittle bodies, *e.g.*, salts, dried pastes, &c.), or crushed in a mortar of brass, steel, or agate, the whole being covered with a cloth to prevent the projection of fragments. The finely-powdered

[1] Pure nickel wire makes excellent triangles for laboratory use. They can now be obtained from most dealers in chemical apparatus.—*Translator.*

material is separated from the coarser particles by means of sieves, and the process is continued until a uniformly impalpable powder has been obtained, which with hard and tough bodies is often a laborious operation.

§ 6. **Dissolving.**—In order to analyse solids in the moist way, they must first be brought into solution. Many substances yield a perfectly clear solution in some solvent, whilst from others different bodies or groups of bodies may be extracted by means of appropriate solvents. Thus water, especially if hot, dissolves sugar, gum, many salts, &c.; alcohol takes up the resins; ether, petroleum ether, chloroform, and carbon disulphide dissolve fats or oils, &c. The solubility of many compounds in any solvent often depends on the temperature maintained, or whether the reaction is acid, neutral, or alkaline. What cannot be extracted by the above-named solvents can often be dissolved in one or in several acids, dilute or concentrated (acetic acid, hydrochloric acid, nitric acid, *aqua regia*, sulphuric acid), or alkalies (ammonia, soda, or potassa lye), often, however, with decomposition. Quite insoluble bodies, silicates, &c., have often to be opened up by fusion with alkalies, the melt being afterwards taken up in water. The solubility or insolubility of a body in one or other solvent generally gives important indications as to its chemical nature. Small specimens are treated with the various solvents in test-tubes, first in the cold and then in heat. Whether anything has been dissolved is ascertained by evaporating the filtrates on watch-glasses, if it is not already indicated by the colour, or the total clearing up of the liquid.

§ 7. **Precipitation, Decanting and Filtration. Drying Precipitates.**—For separating two or more bodies from each other reagents are commonly added to the solution to convert one of the bodies into an insoluble compound, which is then quickly or slowly deposited as a precipitate. Some precipitates (*e.g.*, ammonium-magnesium phosphate) require some hours for their complete formation.

Precipitations are generally effected in beakers, but those in which caustic alkalies or ammonia are required, which attack glass strongly, especially in heat, are performed in porcelain dishes. The question next is to separate the precipitate from the supernatant liquid.

This task is always much facilitated by waiting until the deposit has completely subsided; it is then sometimes possible, without the use of a filter, to pour off slowly the greater part of the supernatant liquid, filtration being only needed for the rest. But in quantitative operations filtration is always needful. There are two known kinds of filters in use, the smooth and the fluted; the latter act more rapidly, but are fit only for qualitative operations, as on them the precipitate cannot be readily washed. At present ready-made filters are so cheap that the chemist does not need to cut and fold them personally. On packets of filters intended for quantitative analyses the proportion of ash in a single filter is indicated; it is generally very small.

The funnels are always of glass, with a section of 60°, and the point is preferably ground off obliquely. The glass to receive the filtrate is placed so that the point of the funnel may touch its side, and may be at the distance of 3 to 5 centimetres distance from the level of the liquid in the lower vessel.

The filter is placed in the funnel in such a manner that it is everywhere in contact, and reaches to a few millimetres from the margin of the funnel. Before pouring in the liquid the funnel is moistened with a few drops of distilled water, or alcohol, ether, &c., according to the liquid to be filtered.

The liquid is poured slowly out of the beaker without stirring up the precipitate, the lip of the beaker having been first greased externally with vaseline at some one spot. The stream is allowed to float down a glass rod, which is held perpendicularly; its point is coated with a small piece of caoutchouc tubing, or a special caoutchouc cap. The filter is filled only up to half a centimetre from the top, but it must be frequently replenished to prevent loss of time. When only the precipitate remains in the beaker, along with a little liquid,

it is washed in the beaker (with water, dilute ammonia, or dilute acid, as the case may be), allowed to deposit again, the washings are poured upon the filter, the precipitate is washed again and poured completely upon the filter, the sides of the beaker being cleansed by means of the caoutchouc wiper and numerous small portions of water. The precipitate collected upon the filter must be freed from the last traces of accompanying soluble impurities by means of water, or some other solvent, cautiously applied with the washing-bottle, until the solvent runs out pure, which must be ascertained in every case by means of suitable tests.

If we wish to obtain only a portion of the precipitate or of the filtrate for further qualitative examination, the required simplifications are self-evident; we break off the filtration as soon as the substance required for investigation has been obtained. Filtration at a reduced atmospheric pressure is rarely needed in hygienic research. For details see § 79 and § 219.

§ 8. If the filtered precipitate has to be dried and weighed, a piece of filter paper is laid over the mouth of the funnel, moistened with the washing-bottle, and the overhanging margin is stroked off with the finger and thumb, so that there remains a closely-fitting round cover, which prevents dust from falling in whilst it does not interfere with drying. When the filter is dry it is carefully lifted out of the funnel, folded together if necessary, and laid in a light, wide-mouthed weighing-glass; the filter and glass are left for fifteen minutes in the drying-closet, allowed to cool in the exsiccator, and weighed. If it is once more placed in the drying-closet for half an hour, and again cooled in the exsiccator, and on weighing is found to have lost in weight not more than 1 milligramme, the drying is regarded as complete, otherwise it is repeated a third time. If the empty filter has been previously dried and weighed in the same glass, the weight of the precipitate is found by subtracting the weight of the empty from that of the full filter.

If the precipitate under determination is not modified by

ignition (*e.g.*, $BaSO_4$), or if it passes into a compound the composition of which is exactly known, from which we can infer the body sought (*e.g.*, $MgNH_4PO_4$ converted into $Mg_2P_2O_7$), we always avoid the use of a weighed filter. We burn the filter along with the precipitate in a porcelain crucible, ignite the remaining mass until the last trace of carbon has been consumed, and subtract the weight of the ash of the filter from the weight which has been ascertained.

If it is suspected that, on burning the filter, incomplete and therefore incalculable processes of reduction have been occasioned by the carbon, *e.g.*, $BaSO_4$ to BaS, certain metallic oxides to suboxides, &c., the precipitate is removed by cautious pressure of the outside of the dried filter, carefully allowed to fall into the crucible (set upon a sheet of glazed paper). The filter, which is emptied as nearly as possible, is rolled up and folded, and burnt separately on the lid of the crucible, which is held cautiously in the flame. Lastly, the ash of the filter is added to the contents of the crucible, which is covered with its lid, ignited for a short time, and let cool in the exsiccator. Exsiccators are well-fitting glass vessels, partly filled with a hygroscopic substance (calcium chloride or concentrated sulphuric acid), so as to form a suitable space in which the crucible may fit without absorbing moisture. One design of exsiccators is shown in Fig. 5*a*. In order to admit several crucibles at once, bell glasses ground so as to fit upon glass plates are much in use. In them is placed a shallow vessel for sulphuric acid, covered with a porcelain lid having apertures for inserting the crucibles. Objects weighed whilst hot appear too light, as they produce ascending currents of air around the scale-pan.

FIG. 5*a*.—Exsiccator.

§ 9. **Hints on Working Glass, Cork, and Caoutchouc.**—Glass rods and glass tubes are cut to the desired length by notching them with a three-square moistened file and breaking them. The thumb nails are placed upon the tube

opposite to the notch, and bending so that the notch is on the convex and the fingers on the concave side of the bend. The wider and thicker the tubes the more difficult is the work, and it is often necessary to file all round and expend a considerable time.

For wide tubes the following method is the best: The tube is wrapped round with two broad wet slips of blotting paper, which are tied firmly in such a manner that a small strip of glass remains uncovered between them. If a narrow hot blast-flame is directed upon this spot the tube generally breaks as desired. An oblique end to a tube is obtained by grinding on a moistened piece of sandstone.

For the greater part of laboratory work easily fusible soda-glass is used. Tubes are bent, always after being carefully dried within and without, by holding them in the luminous flame of a bat's-wing burner, so that a considerable length of the tube can be heated at once. We do not bend until the tube has been softened on all sides by turning it in the flame. It is especially difficult to bend wide tubes neatly. For drawing out tubes we heat with the point of a Bunsen flame, or for thicker tubes the blast-flame, as also for sealing them or for rounding off sharp fractures. Sealing is effected by heating strongly a short piece of the tube as near as possible to the point intended to be closed and turning it round; it is slightly drawn out, the contracted part is again heated and again drawn out, the narrow connection is cut off, and the open end held in the flame until it closes. By holding it for some time in the flame we obtain a solid closure.

Every application of a melting-heat should be preceded by a short warming, moving the tube quickly to and fro in the flame.

Tubes of difficultly fusible potash-glass can only be worked with the blast-flame, but in other respects in the same manner as soda-glass.

Corks must be soft and free from coarse holes; to adapt them to narrow apertures they are trimmed with a fine sharp file. Every cork before use is gently pressed in the cork squeezer. In order to perforate corks we use brass tubes

sharpened below (cork-borers); a complete set of such borers should be kept at hand. The boring should be performed slowly and in a perfectly perpendicular direction; the borers must always be kept sharp by the use of a steel file. If the holes in the corks are too narrow they may be afterwards enlarged by means of a round file. Corks afford air-tight connections only if they are afterwards coated with paraffine; certainty, however, is only to be obtained by means of caoutchouc stoppers. Such stoppers can be ground thinner with emery paper. Their perforation is difficult and tedious even with sharp borers moistened with potassa-lye, but they can be bought with perforations of any required size.

New caoutchouc tubes should be exposed before use to a blast of air to remove the dust of talc, and it is better to boil them for some time in distilled water.

§ 10. **Reagents.**—As useful work can be done only with the purest reagents, they should be procured only from the most eminent makers. Merck of Darmstadt has lately manufactured preparations labelled "*pro analysi*," the purity of which he guarantees.

The following are the most useful reagents (the proportion of crystalline water is indicated where it occurs):—

1. *Acids.*

Acetic acid concentrated, *i.e.*, 50 per cent., sp. gr. 1·06.
Nitric acid concentrated and 1 : 3.
Hydrochloric acid concentrated and 1 : 3.
Sulphuric acid concentrated and 1 : 8.

2. *Bases.*

Liquid ammonia, about 10 per cent. ammoniacal gas, sp. gr. 0.96.
Potassa lye (KOH) and soda lye (NaOH), 1 : 10.

3. *Salts.*

The following are used in 10 per cent. solutions:—
Ammonium chloride (sal-ammoniac), NH_4Cl.

Barium chloride, $BaCl_2 + 2H_2O$.
Copper sulphate, $CuSO_4 + 5H_2O$.
Lead acetate, basic, $Pb(C_2H_3O_2)_2 + 2PbO$.
Lead acetate, neutral, $Pb(C_2H_3O_2)_2 + 3H_2O$.
Magnesium sulphate, $MgSO_4 + 7H_2O$.
Platinum chloride, $PtCl_4 + 8H_2O$.
Potassium bichromate, $K_2Cr_2O_7$.
Potassium monochromate, K_2CrO_4.
Potassium ferricyanide, $K_6Fe_2C_{12}N_{12}$.
Potassium ferrocyanide, $K_4FeC_6N_6 + 3H_2O$.
Potassium sulphocyanide, KSCN.
Sodium phosphate, $Na_2HPO_4 + 12H_2O$.

The following are used in 5 per cent. solutions:—

Ammonium oxalate, $(NH_4)_2C_2O_4$.
Ferric chloride, Fe_2Cl_6.
Mercuric chloride, $HgCl_2$.
Silver nitrate, $AgNO_3$.

Other Liquid Reagents.

Sulphuretted hydrogen water, prepared by saturating cold distilled water, previously well boiled, with hydrogen sulphide.

Yellow ammonium sulphide, which may be bought, or it may be prepared by saturating liquid ammonia with sulphuretted hydrogen and digesting the colourless liquid, $(NH_4)_2S$, with a little powdered sulphur, so as to form the yellow polysulphide.

Alcohol at 96 per cent.

Ether, chloroform, and petroleum ether may be bought.

The following substances (in addition to the solids required for preparing the above solutions) should be kept in glass bottles, corked, or with glass stoppers:—

Ammonium carbonate [$(NH_4)_2CO_3$], ammonium molybdate [$MoO_4(NH_4)_2$], barium hydroxide [$Ba(OH)_2 + 8H_2O$], barium carbonate [$BaCO_3$], borax [$Na_2B_4O_7$], calcium chloride [$CaCl_2$] fused, calcium oxide [CaO], iron sulphide [FeS], ferrous

sulphate [$FeSO_4 + 7H_2O$], potassium iodide [KI], potassium nitrate [KNO_3], potassium chlorate [$KClO_3$], potassium permanganate [$KMnO_4$], magnesium chloride [$MgCl_2$], sodium chloride [NaCl], sodium hyposulphite = sodium thiosulphate [$Na_2S_2O_3 + 5H_2O$], soda-lime, sodium carbonate [$Na_2CO_3 + 10H_2O$], potassium sodium tartrate [$C_4H_4KNaO_6 + 4H_2O$], oxalic acid [$C_2O_4H_2 + 2H_2O$], strontium nitrate [$SrNO_3$], tartaric acid [$C_4H_6O_6$], zinc free from arsenic; brucine, diphenylamine, indigo, grape-sugar, milk-sugar, starch, paraffine, vaseline, animal charcoal.

The more complicated reagents, and those rarely employed, are described in the text, and may be found in the index. For indicators see § 26.

2. The Spectroscope.

§ 11. The spectroscope ranks among the most useful instruments of the hygienist. It is well known that incandescent metallic vapours emit only light of one or of several definite wave-lengths, hence their spectrum consists of one coloured line—or of several such—characteristic for each metal. Thus sodium, potassium, lithium, calcium, barium, and many other metals may be recognised in the minutest traces if we evaporate their more volatile salts (*e.g.*, chlorides) in the flame of a Bunsen burner (emission-spectrum). The hypersensitiveness of this reaction deprives it, however, of practical hygienic interest, as we are here concerned only with ponderable quantities of such matters.

Much more important in hygiene is the examination of absorption spectra. Kirchhoff proved that every substance as vapour or in solution absorbs exactly those rays of light in the spectrum which it emits in the state of an incandescent vapour (if, indeed, it can be brought into this state without dissociation). The absorption at certain parts of the spectrum, which is extremely characteristic for individual compounds, gives rise to dark bands, and enables them after to be recognised in quantities which are not readily demonstrable by

other means, *e.g.*, magenta, hæmoglobine, carbon monoxide, &c., every trace of which concerns us if found in certain places.

A simple spectroscope is constructed as follows: A glass prism *p* stands on a brass pillar, to which are secured three horizontal brass tubes fitted with lenses. The tube *c* is fixed, whilst the two tubes *a* and *s* are placed in separate sheaths on a perpendicular rotatory axis, and can therefore be made to revolve upon it independently of each other.

Into the tube *c* (collimator tube) there falls through a narrow slit a bright bundle of rays upon the collimator lens fixed at the other end of the tube, from which it issues in

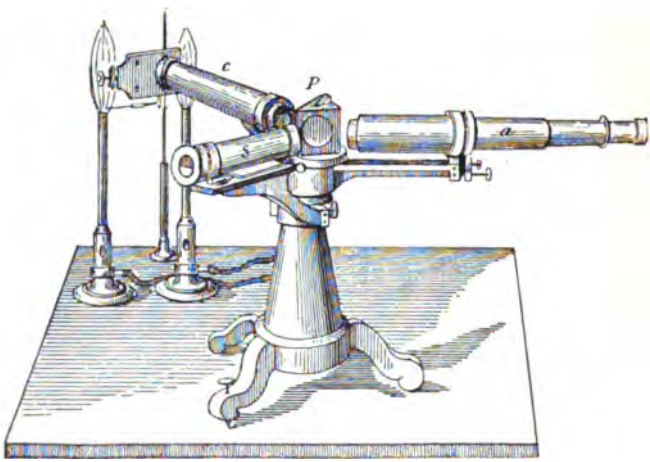


FIG. 6.—Spectroscope.

parallel rays. In the track of these parallel rays is introduced the strongly refractive prism *p*, in such a manner that the deflection of the yellow rays is a minimum. An objective image of the spectrum is projected by the object-lens of the telescope, *a*, which is adjusted for parallel light, and can then be examined as magnified six to eight times by the lens serving as eye-piece. A thread cross is fixed in the eye-piece.

Simultaneously with the spectrum we see the image reflected by the prism of a fine scale photographed upon glass and placed at the extremity of *s*; it is illuminated by a gas-flame (Fig. 7).

As a source of light we use the light of the sun, or, more commonly, an argand burner, but preferably a flat petroleum burner. In the latter cases the light is emitted by incan-

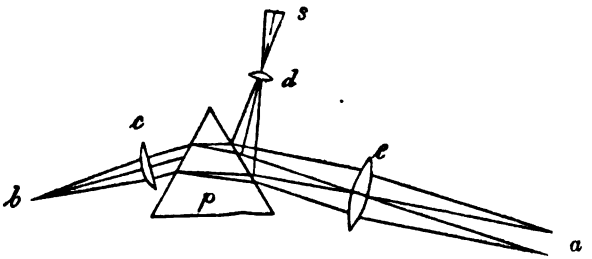


FIG. 7.—Diagram of the Spectrum Apparatus. *b*. Source of light; *s*. scale; *c*. lens of the telescope which projects the image.

descent solids (carbon), and therefore contains all kinds of rays, and gives a continuous spectrum.

§ 12. **Arrangement of the Apparatus.**—The adjustable slit is made very narrow, and there is placed before it a sodium flame, *i.e.*, a Bunsen flame, into which there projects a globule of sodium chloride or of soda on a platinum wire. The telescope *a* is taken out and adjusted at an open window for a distant point (a lightning-rod or a tree). This is effected by displacing the eye-piece. The prism is then removed, and the telescope *a* is returned to its place and turned in on the prolongation of the collimator tube *c*, the draw-tube of which, carrying the slit, is so regulated by drawing out and pushing in until the slit can be seen quite sharply, with any existing imperfections of its edges. The slit then lies, as it ought, exactly in the focus of the collimator-lens. The prism is now returned to its place,[1] and the telescope *a* is turned so that we can again see through it the deflected image of the slit. A moderately bright flame is lighted in front of the scale telescope, and the latter is turned (letting the flame constantly follow) until the reflected image of the scale appears in the telescope. Degree 50 of the scale is made to coincide with the sodium line, or with the image of the slit.[2]

[1] The position of the prism is generally determined once for all by a depression in which its base is set. This position must be such that yellow light undergoes the minimum of deflection, so that the image in the telescope, on turning the prism in one or the other direction, is displaced each time towards the same direction.

[2] The scale is adjusted correctly by drawing out and pushing in until its image lies in the same plane with that of the slit. It is correct, therefore, when, on moving the head, slit and scale are no longer displaced paralactically to each other. But without fulfilling this condition, the slit and the scale may both be accurately seen by a different exertion of accommodation.

The slit consists of two sharply-defined steel edges, the one fixed and the other movable; a slit of 0·1 *mm.* in width is generally used. For many purposes it is very useful to project the spectra of two sources of light over each other by means of the same slit. This is effected by introducing in front of the lower part of the slit a small comparison prism, as shown in Fig. 8. Hereby the light of a lateral flame is thrown into the collimator tube by total reflection. There then appear in the telescope two spectra, similar colours of which lie exactly above each other. The upper spectrum is derived from the lower half of the slit (source of light *A*) if it is covered by the comparison prism, as the telescope inverts the image, whilst the lower spectrum is derived from the source of light *B*. The main prism is enclosed in a metal case. The observations are undertaken in a somewhat darkened room, in order not to be disturbed by dazzling in the appreciation of delicate absorption bands.

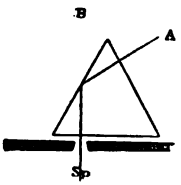


FIG. 8.

§ 13. If we wish—which is rarely needful—to search for metals by means of their spectral lines, the substance is dissolved in a drop of the purest hydrochloric acid, so as to obtain chlorides. We dip into the liquid a loop of platinum wire well cleansed by ignition (ignited so long that the flame of the Bunsen burner no longer shows the yellow sodium colour), fixed in a handle, and again introduced into the Bunsen flame, noting the position of the most important lines, which are identified either by means of tables, or, preferably, by direct comparison with the spectral lines of the various pure alkalies, alkaline earths, &c., which come in question. We must attend only to the more persistent lines, as the particles of dust suspended in the air may occasionally introduce various foreign matters. For examining the absorption spectra of coloured liquids, they are preferably placed in small bottles with their sides ground parallel, or in small boxes of mirror-glass. The images are less fine if we use test-tubes, those only being suitable which have smooth sides and are free from flaws. The glasses are fixed on a support and placed directly before the slit, moving them slightly until any absorption perceptible becomes most distinct. In front of the comparison-prism may be set a second glass with a comparative solution and a second gas-flame.

Many coloured liquids give merely an abbreviation of the spectrum. Yellow and red liquids transmit little blue or violet, the more refrangible end of the spectrum is therefore darkened, whilst blue liquids extinguish the red end of the spectrum to some extent. Along with these less characteristic terminal absorptions—which, of course, must be duly noted—many substances give dark stripes or bands in the spectrum, which may be narrow or broad, sharply bounded, or with fading outlines. These bands demand our greatest attention. If we see in the spectrum a broad dark band, *e.g.*, from C to D, we cautiously dilute the liquid by degrees, and notice successively the appearances presented, whether the broad band before its disappearance is resolved into several narrow bands, &c.

§ 14. The position of the bands observed is indicated by many authors only in an approximate manner, as, *e.g.*, "band between D and E," or, "two narrow bands between F and G, nearer to the latter." Somewhat more accurate is the following manner of expression, "band at D $\frac{1}{3}$ E," *i.e.*, if the space between D and E is divided into three parts the band lies in the first of these divisions.

The statement may be made more precise by means of the scale-tube. If we place D at 50 we need merely state at what point of the scale a few other well-known lines fall, *e.g.*, B at 31, E at 70, F at 89, in order to give, by saying that a band extends from 63–67, a perfectly definite account which may be verified by others. It would mean, if we divide the space from D to E into twenty parts, the band extends from the 13th to the 17th degree. For determining the position of the main lines, we may use sunlight (*i.e.*, Frauenhofer's lines), or characteristic metallic vapours, obtained as in § 13. The line A corresponds to the only predominant potassium line, B to the strontium line situate most to the red side, D to the sodium line, F to the extreme blue barium line.

Really exact measurements of the position of bands can be effected only with especial apparatus (spectrometers), which cannot be described

here. As a measure we use the wave-length at the point in question, expressed in millionths of a millimetre :—

A	= 760·4	E	= 526·9
B	= 686·7	F	= 486·1
C	= 656·2	G	= 430·7
D_2	= 589·5 } With small apparatus these	H	= 396·8
D_1	= 588·9 } two appear as a single line.		

The accuracy of the statement of the place of absorption bands is rendered more difficult by the circumstance that they differ in breadth with increasing concentration and thickness of stratum. If it is not possible to give the degree of concentration, we must be content to characterise the bands separately for weak, medium, and strong solutions, a procedure which is defective but mostly sufficient. Others

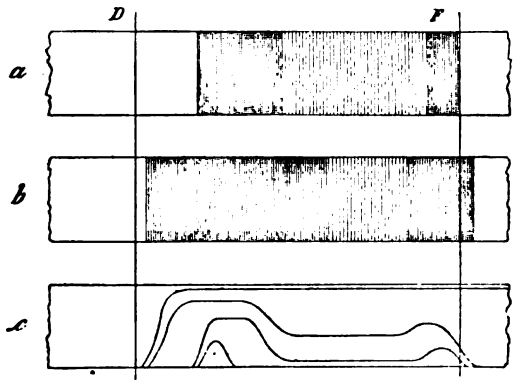


FIG. 9.

do not describe the bands, but express the gradual increase of the absorption as stronger and stronger solutions are used. An example shows this distinctly (Fig. 9).

a is the spectrum of a solution of magenta 12 *mm.* in thickness, containing $\frac{1}{2}$ *mgrm.* magenta in 100 *cc.* of alcohol at 50 per cent.

b the same when 1 *mgrm.* magenta is dissolved.

In *c* the lowest curve expresses the absorption under the above conditions when $\frac{1}{4}$ *mgrm.* is used; the second curve corresponds to the figure *a* ($\frac{1}{2}$ *mgrm.* magenta); the third curve corresponds to the figure *b* (1 *mgrm.* magenta); the outer curve

expresses the absorption under the above conditions when 2 *mgrm.* magenta are used.

§ 15. For preliminary researches, or for most practical purposes, small "direct vision" spectroscopes are generally sufficient. By a suitable combination of flint and crown glass we can obtain a system of prisms that does not deflect the mean rays (yellow), but disperses the other colours strongly (flint glass disperses strongly, but refracts slightly; crown glass acts inversely).

The telescope and the collimator may thus become a single draw-tube, which is held to the eye, whilst the slit is turned towards a white cloud or a gas-flame. The eye-piece is then moved until the spectrum reaches its maximum distinctness, or, if sunlight is employed, until the Frauenhofer lines are seen, when the absorbing liquid can be held direct before the instrument. The position of the bands can be determined only by means of a comparative prism. Vogel's universal spectroscope is especially approved of; its full description is found in H. W. Vogel's "Spectral Analysis of Terrestrial Substances," 2nd edition, Berlin, 1887, in which will be found the entire material hitherto collected in spectroscopic investigations with especial reference to practical requirements. H. Kayser's "Text-Book of Spectroscopic Analysis," Berlin, 1883, treats chiefly of the theory of spectral analysis and of metallic spectra.

Microspectroscopes have also been constructed for the spectroscopic examination of the colours of microscopic objects, as also polarising microscopes, which, however, are of little importance in hygiene. See Schellen's "Spectrum Analysis," translated by Jane and Caroline Lassell, London, Longmans & Co., 1885; also Behrens, *Hilfsbuch für Micros. Untersuchungen*, Brunswick, 1883, and Behrens, Kossel, and Schiefferdecker, *Das Microscop und die Methoden der Micros. Untersuchungen*, Brunswick, 1889.[1]

For quantitative determinations hygiene has not yet made use of the spectroscope, though in some cases it would certainly prove applicable; but for every new substance to be determined it would be necessary to

[1] See also "Spectrum Analysis as Applied to Microscopical Observations." W. T. Suffolk, F.R.M.S. London, Browning, 1873.

draw up a table, and the operator must have the costly apparatus at his disposal. A good outline of the method is given by Kayser, *op. cit.*, p. 213, and H. W. Vogel in the article *Spektralanalyse*, in Dammer's *Lexikon der Verfälschungen*. For more special studies is required: G. Krüss and H. Krüss, *Kolorimetrie und Quantitative Spektralanalyse*, Hamburg, 1891.

3. Determination of Absolute and Specific Weight.

§ 16. The use of coarser balances, such as are used by grocers and apothecaries, is well known. The "tare balance" of the apothecary, which turns with 5 to 10 *mgrm.* when loaded with 1 *kilo.*, is very serviceable in weighing heavy objects. The plate balances are for weighing animals, large samples of soil, bottles for the determination of carbonic acid, &c. When loaded up to 10 *kilos.* they indicate from ½ to 2 *grm.*

The use of a fine analytical balance is best learnt by personal instruction, but the chief rules must not be omitted here. The balance must be kept always absolutely dry, being wiped with a fine cloth or leather, which must be kept in the drawer of the balance case; the glass case in which it is enclosed must always be kept shut when not in use, and also at the end of each weighing, when the milligrammes are being ascertained. The balance stands (being never moved) upon a solid table (preferably a wall-bracket), and is kept perfectly horizontal by means of levels.

The most important properties of a sensitive balance are: The beam is long,[1] but is at the same time rendered light by the structure shown in Fig. 10; at its middle it supports a long steel index, *f*, which plays in front of an ivory scale. The vibrations of the beam are shown by the movements of the index in front of the scale. In the middle of the beam, *a*, there is a sharp steel knife-edge, *b*, pointing downwards, which during weighing rests on a steel plate on the upper

[1] Recently balances have been made with short arms. These, in consequence of the reduced length of the beam, do not oscillate for so long a time, but are so constructed as to be very sensitive. These short-armed balances have the further advantage that the beam is less deflected by heavy loads, so that they are almost equally sensitive for small and large weights.

surface of the support, or, as in our figure, upon the steel column *c c* (Figs. 10 and 11). The scale-pans are also very light, and hang from steel knife-edges on three-edged steel bearings.[1] The centre of gravity of the beam is close below the turning-point; a screw in the middle of the beam allows its position to be modified. To prevent the knife-edges from wearing, the pans, when at rest, are supported by small

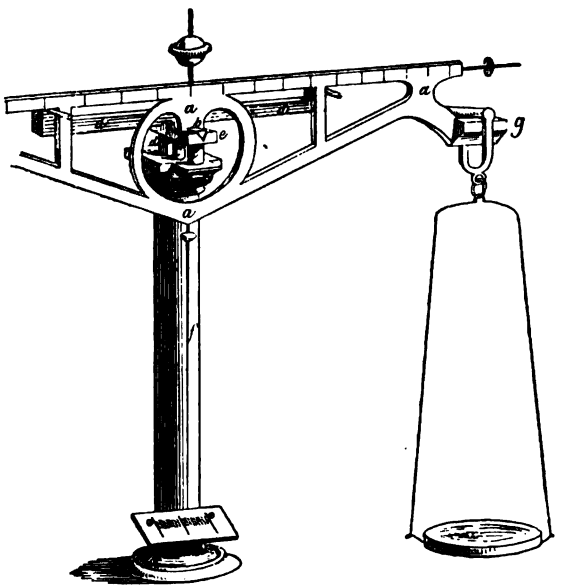


FIG. 10.—Balance at rest.

tables. Further, the beam is lifted by a kind of fork, *d d* (Figs. 10 and 11); the knife-edge is raised separately at the same time by the trough *e*. The fork and the trough are both secured to the vertical rod *d*, which moves in the balance column *d* behind *c c* (Fig. 10). The fork is lowered by turning a lever, the milled head of which projects between the drawers of the balance case. The beam is thus liberated, the knife-edge comes upon the bearings, and at

[1] The knife-edges and their bearings are preferably made of agate, so as to be unaffected by acid vapours.—*Translator.*

the same time the tables underneath the scale-pans are lowered, so that the latter hang freely.

§ 17. If a weighing is to be effected, the clean condition of the scales is first ascertained, and any rider (see below) which may have been forgotten is removed. The beam is then raised and the pans liberated by turning the handle, and the oscillations of the beam to the right and the left are then compared. After one or two vibrations the preponderance must be equal on each side. If this is not the case, the true position of equilibrium must be determined by an unequal number of readings. If the balance inclined

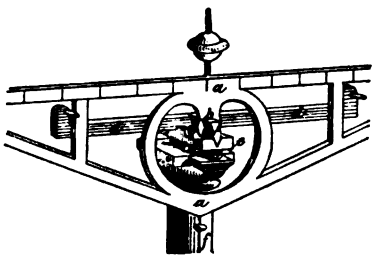


FIG. 11.—Beam in motion.

first to the right	9·0,	then to the left	6·3
then „	„	8·1	„	„	5·4
„ „	„	7·3			

the zero-point is $24{\cdot}4 \div 3 = 8{\cdot}1$ $11{\cdot}7 \div 2 = 5{\cdot}8$
$8{\cdot}1 - 5{\cdot}8 = 2{\cdot}3$. As the balance, therefore, turns 2·3 degrees too far to the right, the zero-point lies at $2{\cdot}3 \div 2 = 1{\cdot}15$ to the right. A slight movement of the screws fixed laterally at the ends of the beam allows us to fix the position of equilibrium corresponding to the middle of the scale; the tyro, however, should beware of frequent screwing.

After this preliminary trial the object to be weighed is always laid on the left-hand scale, and on the right-hand scale a weight (taken from the weight-box), which is supposed to be approximately equal to the object, or slightly heavier. If the beam and pans are then gently liberated by turning the handle, it is at once seen which side preponderates, and the vibration is at once arrested. This must be done cautiously and slowly; rapid stoppage may easily damage the balance. When the weight has been approximately ascer-

tained—say within 1 *cgrm.*—the checking mechanism is for the moment entirely removed, so that the balance may come to rest. In weighing[1] it is best to proceed quite systematically, as will be understood from the following example:—

Suppose we have to weigh a porcelain capsule, the weight of which, unknown to the operator, is 24·6432 *grm.* The following weights are put on in succession on the right-hand scale pan:—

20 too light,
20 + 10 too heavy,
20 + 5 too heavy,
20 + 2 too light,
20 + 2 + 2 too light.

We therefore know it is heavier than 24 but lighter than 25. We proceed:—

24 + 0·5 too light,
24 + 0·5 + 0·2 too heavy,
24 + 0·5 + 0·1 too light.

It is therefore heavier than 24·6 but lighter than 24·7.

24·6 + 0·05 too heavy,
24·6 + 0·02 too light,
24·6 + 0·02 + 0·02 too light.

It is therefore heavier than 24·64 but lighter than 24·65.

§ 18. The completion of the weighing with milligrammes and their fractions is effected, not by laying upon the scale

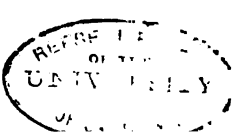
---

[1] The general sets of weights contain gilt brass weights down to 1 *grm.*, and platinum weights for the fractions of a gramme, being generally

1 weight at	50	5	0·5	0·05	0·005
2 „	20	2	0·2	0·02	0·002
1 „	10	1	0·1	0·01	0·001

The series 10, 1, &c., is more rarely in duplicate, and that at 20, 2, &c., only single. The weights must be lifted with an ivory-pointed forceps only; the points of the platinum weights must lie upwards and be turned to the right; the weight or rider must never be left lying on the scale-pan. Recently, fractions of the gramme are made of aluminium; they are large and convenient. For checking the weights, the sensitiveness of the balance, &c., see Kohlrausch.

[In many respects the arrangement adopted for English weights is more convenient than that just described. The weights down to 10 grains are of gilt brass, and represent 100, 60, 30, 20, and 10 grains. For grains the weights are made of platinum wire bent so that their value can be seen more easily than if they were plates stamped with a number. They are, 6, 3, 2, 1; and for tenths and hundredths of a grain bent aluminium wires, 0·6, 0·3, 0·2, 0·1, 0·06, 0·03, 0·02, and 0·01. By this arrangement any required number can be made up with fewer pieces than on the French system.—*Translator.*]



milligramme weights, which are inconvenient from their smallness, but by means of a so-called "rider." A wire hook weighing exactly 10 *mgrm.*, if placed on the middle of one-half of the beam, will produce only half the effect which it would have if laid on the pan, *i.e.*, 5 *mgrm.* The right half of the beam is therefore divided into ten parts, and by simply displacing the rider with a sliding-rod we may weigh from 1 to 10 *mgrm.* Fractions of a milligramme may be estimated or read off by graduating the right arm of the beam in 100 parts. If the rider falls down, it must be restored to its place with forceps.

When both scale-pans are brought into equilibrium, the result is read off deliberately and carefully, since nearly all errors in weighing, and even the majority of serious analytical errors, are simply faults in reading off. This reading is always done doubly. We first add together the values of the pieces which are wanting in the box, and note down the weight; we then add up the weights as we take them from the scale, examining them carefully as we lay them in their places. If the two sums do not correspond, the weights must be returned to the scale-pan.

In almost all weighings we have to weigh several times. A vessel is first weighed empty, and then along with the substance; the weight of the latter being known by the difference. Those who have to weigh frequently should provide themselves with light, wide-necked glass vessels of different sizes with light ground glass stoppers, the weight of each of which is marked on it with a diamond. These are to be used as weighing-glasses. Each glass must be very carefully cleansed before use; even the contact of moist fingers, &c., may cause an increase of weight up to 5 *mgrm.*

§ 19. **Determination of Specific Gravity.**—By the specific gravity of a body we understand the proportion of its density to the density of water—or the weight of 1 *cc.* of the body, as 1 *cc.* of water weighs 1 *grm.* One *cc.* of water, indeed, weighs exactly 1 *grm.* only at 4°; nevertheless, the specific gravity of solids only is referred to water at this temperature. Liquids are generally weighed at 15°, and their specific gravity is referred to water at this temperature;

more rarely (*e.g.*, Sohxlet's areometer) 17·5° is assumed as the normal temperature.

Solids.—If the substance has a known volume, it is rarely necessary to divide its absolute weight by this volume; an unknown volume is ascertained according to § 37, either by calculation or experiment.

§ 20. The determination of the specific gravity of liquids is of far greater importance for hygiene. It may be effected in different manners.

1. *With the Ordinary Balance.*—We weigh a volume of the liquid, measured as exactly as possible and not too small, say from 5 to 50 *cc.* For this purpose there is generally employed the so-called pycnometer (πυκνος, dense), small bottles with a ground hollow stopper ending in a capillary tube. The bottle is filled after removing the stopper, so that a convex layer of liquid rises above the mouth. If the stopper is now inserted its hollow is entirely filled with the liquid. The apparatus is then most cautiously dried. Accurate determinations must be made with liquids at 15°; to facilitate this the best pycnometers are fitted with thermometers melted into the stopper and dipping into the liquid.

FIG. 12.—Pycnometer.

For determining a specific weight these weighings are performed:—

1. Pycnometer empty and dry $= a$.
2. Pycnometer filled with distilled water at 15° $= b$.
3. Pycnometer filled with the liquid in question at 15° $= c$.

We have then d (the specific gravity) $= \frac{c-a}{b-a}$.

2. *With Hydrometers (Areometers).*—The most convenient and general method. A float of glass (those of vulcanite have not come into use), ballasted below with mercury or fine shot, and of known form, sinks into a liquid until the submerged portion of the instrument is equal in weight to the volume of the liquid which it displaces; hence deeper in light liquids, and less deep in heavier liquids. If a

hydrometer is immersed in a series of solutions of sodium chloride, the specific gravity of which has been accurately tested by method 1 or 3, and, *e.g.*, the points 1·000, 1·010, 1·020, and 1·027 are thus determined, the interval 1·000–1·010 is divided into 10 degrees, 1·010–1·020 into 10 degrees, and 1·020–1·027 into 7 degrees. The apparatus is very good and useful if the degrees thus obtained are equal among themselves. Areometers purchased are checked at numerous points in the manner just described, and a table of corrections is then drawn up. The liquids must have a fixed temperature (generally 15°), and they are poured for examination into cylinders of such a width that the hydrometer does not touch

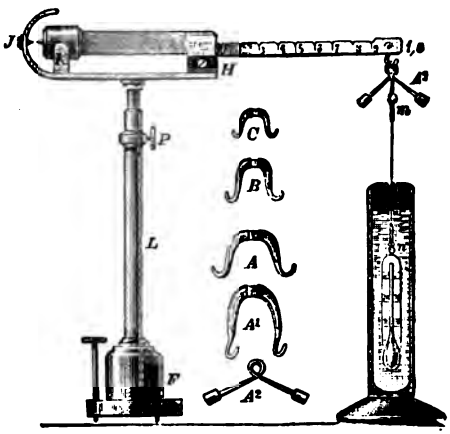


FIG. 13.—Westphal Balance.

the side of the cylinder at any point. When reading the figure we must remember that aqueous liquids rise up against the stem of the instrument in consequence of capillarity; hence we must beware of reading off too high a level, *i.e.*, specific gravity, but take the true level of the liquid.

3. *With the Mohr Westphal Balance.*—Just as we may infer from the depth to which one and the same float sinks into different liquids their respective specific gravities, the same end may be reached by loading one arm of a balance (to which a float is fixed) with different weights, so that it may always sink to the same depth into a cylinder filled up

to a mark with the liquid in question. The denser the liquid the more weights must be attached, since the upward impulse is so much the stronger. The beam is divided into 10 degrees, with a notch at each degree. We use three kinds of weights:—

$$A = A_1 = A_2;\ B = \tfrac{1}{10}\ A;\ C = \tfrac{1}{100}\ A;\ D = \tfrac{1}{1000}\ A.$$

We weigh first with the A weights. If the liquid is heavier than water, A_2 is placed at the end of the beam, and in addition an A weight at a notch where it indicates tenths; if the liquid is lighter than water, A_2 is not used. If we have weighed accurately to tenths of a gramme, *i.e.*, if A is placed at 3 the weight is too light, and if at 4 it is too heavy, we seek to place B, signifying the second decimal place, at a notch in such a manner that the weight is rather too light. C is then attached so that the balance is at rest, and the point of the short arm of the beam is opposite to the middle point of the metal arch (Fig. 13, J). In a delicate Westphal balance a fourth weight, D, is provided, with which the fourth decimal place of the weight may be determined. Figure 14 gives examples showing all further explanations.

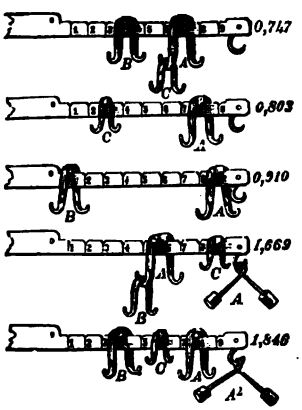


FIG 14.

In every determination the float at its zero-point should be immersed up to the twisted part of the platinum wire by which it is suspended. When the balance is set up we determine once for all the level up to which the small glass is to be filled, so as to fulfil the above condition, and mark it with a writing diamond.

The specific gravity of fats and waxes is determined by casting them in the shape of balls, and determining the specific gravity of a mixture of water and alcohol (lighter than 1·0), or water and glycerine, or, if needful, a solution of sodium chloride, in which the ball may exactly float. The balls are obtained by dropping the liquefied fat from a

height of 2 to 3 *cm.* into cold alcohol of the strength of from 60 to 90°, contained to the depth of 2 *cm.* in a glass capsule with a flat bottom. The globules, which are almost perfectly round, are introduced by means of a spoon into the liquid in the glass. By pouring into the glass a mixture of water and a little alcohol, or alcohol and a little water, or by suitable mixtures of glycerine, we obtain a liquid of the desired specific gravity. When the globules swim quietly in the midst of the liquid without rising or sinking on agitation, the liquid is filtered through wool or glass wool, and determined with the Westphal balance. See Hager, *Pharm. Central Halle*, xx. 13. The specific gravity of fats is often determined at 100°. Compare Butter.

4. Principles of Quantitative Analysis.

§ 21. **Introductory Remarks.**—A beginner, at any rate, should never omit to effect quantitative analyses in duplicate, as a check both upon himself and upon the method. Duplicate work takes at most one and a half times as much time as a single operation, and it is a great gain in the way of accuracy. If the check analyses agree fairly, we take the mean of the two. If they do not agree, nothing remains but to make two more determinations. Carefully marking all vessels with the diamond, written paper labels, or writing with pencil on a ground part of the glass, is indispensable. Coloured pencils write very well, especially upon capsules, &c., which have been gently heated.

For the recognition of a substance it is generally sufficient (if necessary after some preliminary operations) to observe the precipitate, or the change of colour which ensues on the addition of suitable reagents. But a quantitative determination requires more circumstantial work.

Chemistry employs two main methods for quantitative determination: (1) the gravimetric method; (2) volumetry; on which follow (3) optical analysis, *i.e.*, colorimetry, quantitative spectrum analysis, and (4) determination by the polarising apparatus.

1. Gravimetric Analysis.

§ 22. The simplest case of gravimetric analysis is when the solvent of a substance is expelled by heat and the dry residue is weighed. In other cases (*e.g.*, the determination of water), the quantity of the substance sought for is inferred

from the loss of weight by removal of water on drying. A very simple method is also when the substance sought for (we will call it x) is removed from a mixture by some solvent, and after the removal of the solvent it is then weighed as such. Thus, *e.g.*, all fat (and save traces of other bodies, fat only) is extracted by ether from a piece of meat to be analysed. After the expulsion of the ether, pure, ponderable fat remains.

In general, however, the substance x is obtained not as such, but only in the state of *a compound of characteristic properties.* Thus we generally determine sulphuric acid as barium sulphate, copper as copper sulphide or as metal, silver as silver chloride, phosphoric acid or magnesium as magnesium pyrophosphate, &c. We prepare these compounds because they themselves, or the compounds from which they are derived, by reason of their peculiar properties, especially their relations of solubility, admit of being separated by filtration from other substances which are simultaneously present. As a matter of course, after these bodies have been separated, dried, and weighed, we must ascertain from their quantity the quantity of the substance sought for (*e.g.*, sulphuric acid, or barium, or copper), which is easy, as chemical compounds are always composed in exactly identical manners, according to their atomic weights.

If we here dispense with everything theoretical, the atomic signs in chemical formulæ tell us *what elements and what proportions of each are present in a compound.*

Thus, silver chlorine, AgCl, represents a compound in which an atom of silver is united with an atom of chlorine, that is, 107·7 parts by weight of silver and 35·4 parts by weight of chlorine. In other words, 143·1 *grm.* silver chloride contain 107·7 *grm.* silver and 35·4 *grm.* chlorine, whence it is easily calculated that in 100 parts silver chloride there are always contained 76·37 per cent. of silver and 24·73 per cent. of chlorine. In barium sulphate, $BaSO_4$, there exist, along with 136·9 *grm.* barium, 32 *grm.* sulphur, and $4 \times 16 = 64$ *grm.* oxygen, or it contains 58·8 barium, 27·5 sulphur, 13 per cent. of oxygen. Hence 136·9 *grm.* $BaSO_4$ corresponds to 98 *grm.* $H_2SO_4[(2·1) + (1·32) + (4·16)]$, or 80 *grm.* $SO_3[(1·32) + (3·16)]$.

For the determination of a substance it is indifferent in what compound it is isolated if such compound is perfectly

separable, admits of drying and weighing without decomposition, and if its composition is exactly known.

In other cases we dispense with obtaining the substance free or in a compound for weighing, and are satisfied with weighing the product obtained by the action of the substance upon some other, and then concluding from the product the quantity of the body sought for.

Thus, in the ordinary method of determining sugar, we weigh the copper which the sugar precipitates from an alkaline solution of copper sulphate in the state of cuprous oxide. The weighed quantity of copper gives quite accurately the quantity of sugar originally present. Compare § 217.

The common character of all these procedures is that to an unknown quantity of a substance x there is added an unknown and generally excessive quantity of a reagent, and that x is either directly found or calculated from the final product of the reaction. Gravimetric analysis, therefore, always presupposes a delicate balance.

2. The Method of Titration, Volumetry, Volumetric Analysis.

Although a comprehensive hygienic investigation without the balance is impossible, volumetry allows of at least many important researches without necessity for the constant possession of a balance.

The volumetric method depends upon the following principle: In order to convert a compound, x, existing in solution into some other, y, there is required a quantity of reagent z proportional to the quantity of x. If we know z, as well as its proportion of the reagent, we can calculate x.

As it is seen, the performance of such a determination (titration) requires the following conditions to be fulfilled:—

1. The substance under examination must exist in clear solution in a liquid miscible with the liquid reagent. We use almost exclusively aqueous, very rarely alcoholic, solutions.

2. We require a reactive (titration) liquid, of an exactly

known value (standard), for preparing which we certainly require a balance.

3. We often need a special reagent (indicator) in order to ascertain when we have added a sufficient quantity of the standard liquid to effect the complete transformation of x.

If these three conditions can be fulfilled titration is possible, and its advantages in comparison with the gravimetric method are:—

(1.) For each method of titration only one or a few standard solutions are required, which, in case of need, may be obtained by purchase; thus a number of investigations are practicable even for an operator who does not possess a balance.

(2.) The method is exceedingly expeditious, certain, and cheap; and in many cases—especially when the determination of small quantities is concerned—even more accurate than gravimetry.

For these reasons every one ought most carefully to make himself acquainted with the volumetric method.

§ 24. In the execution of the method we require, above all things, a solution of the reagent of a known strength. In the simplest case it is obtained directly by weighing out a substance upon a watch-glass and dissolving it in distilled water. For the preparation of the solution it is best to take a measuring-flask, *i.e.*, a long flask with a mark on the neck,[1] holding 1000, 100, 50, &c., cubic centimetres. It is set on a sheet of glazed paper; in its mouth there is put a small narrow glass funnel, the weighed substance is placed upon it (but little falling through on account of the narrowness of the neck), and cold water (or hot water in the case of slowly soluble substances) is slowly poured into the funnel; the watch-glass, and after the substance is dissolved the funnel, being rinsed out. If a few granules of the substance have

[1] Such flasks have generally two marks. The lower, "influx mark," shows the level of a litre poured in; this mark is used in preparing solutions in the flask. The higher, "efflux mark," indicates how high the flask must be filled in order to pour out a litre. It is used in measuring several litres into a larger vessel.

fallen upon the paper, they are collected by means of a feather, and added to the contents of the flask. The flask is then shaken up well, and when everything is dissolved and cooled down to 15° it is filled up to the mark.

In other cases the standard solution cannot be prepared directly, because the substance to be dissolved cannot be obtained so pure, so undecomposed, so dry, &c., as is neces-

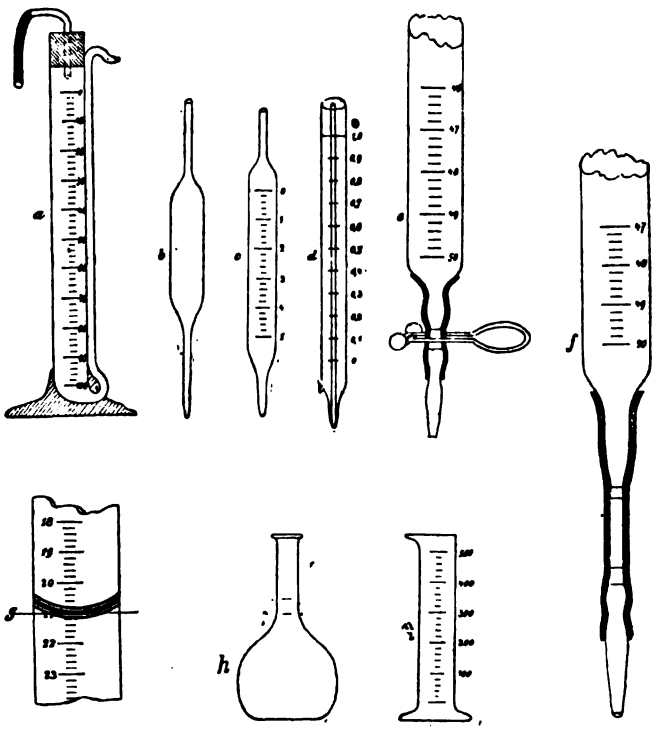


FIG. 15.—Apparatus for Titration.

Explanation.

a. Gay-Lussac's Blowing-burette.
b. Filling Pipette.
c. and *d.* Measuring Pipettes.
e. Mohr's Burette with Pinch-cock.
f. Burette with Bunsen's Stopper.
g. Reading off Level of Liquid.
h. Measuring Flask.
Efflux Mark.
Influx Mark.
i. Measuring Cylinder.

sary for preparing an accurate solution. Hence we must have recourse to various stratagems and circuitous methods.

Thus, *e.g.*, a potassa-lye containing 56 *grm.* potassium hydroxide per litre cannot be obtained by weighing out 56 *grm.* of potassium hydroxide and dissolving it to 1 litre, as it can never be procured free from impurities. But we dissolve, say, 65 *grm.*, and dilute it slowly until 10 *cc.* exactly neutralise 10 *cc.* of oxalic acid of the strength of 63 *grm.* oxalic acid per litre. For 63 *grm.* oxalic acid exactly neutralises 56 *grm.* potassium hydroxide, as follows from their molecular weights.

The standard solution is placed in a tube graduated into tenths of a *cc.*, and known as a burette (Fig. 15), rather drawn out below, and provided with a caoutchouc tube and a glass point, and closed by means of a pinch-cock. The closing may also be effected by means of a short piece of glass rod rounded at the lamp, which completely blocks up the caoutchouc tube (Fig. 15 *f*). By raising a perpendicular fold above the glass rod, the liquid can be run out conveniently and exactly, and the flexible tube is not injured by the pinch-cock. Burettes with glass cocks are suitable for liquids which attack caoutchouc (*e.g.*, strong alkaline lyes), but they are costly, and rather difficult to manipulate. The burettes are fixed vertically, and filled up to a certain mark after all air-bubbles have been expelled from the point by repeated energetic opening of the cock, or even by momentarily turning the cock and compressing the flexible tube. In reading off a burette, we always take the lowest level of the dark meniscus of the liquid; only in very dark-coloured liquids, where the lowest point is not distinct, we read off the upper. The eye must always be at the level of the liquid to be read off. When reading off, we always observe by looking through the burette towards a light surface.

We have now a volume of the liquid in question measured off exactly by means of a suction-pipette,[1] 10 or 100 *cc.*, into a beaker: the indicator is added if required, and the titration begins. The use of pipettes is very easy; its lower

[1] Liquids which are dangerous if taken into the mouth (strong acids and alkalies) are sucked up by drawing a caoutchouc tube over the mouthpiece of the pipette, and sucking at it.

end is placed in the liquid, whilst the upper end is held in the mouth. When the pipette has been sucked full, about 1 *cm.* above the upper mark, the top is rapidly closed with the tip of the forefinger (not the thumb) after it has been closed for a moment, if very full, with the point of the tongue above and with the finger below. The contents of the pipette are run out in small drops down to the mark by means of gentle rotatory movements with the forefinger. It is then firmly closed, the point is wiped externally with the clean hand, and the contents of the pipette are then run as completely as possible into the beaker. If drops remain adhering to the pipette, they must be emptied partly by closing the pipette with one finger and holding it with the palm of the other hand: the warm air within expels the remaining contents. Or the pipette may be emptied by rubbing its point against the glass side of the beaker with due care. The operator must never blow into a pipette.

Pipettes which have a second mark at from ½ to 1 *cm.* from the point, showing to what depth the pipette must be emptied so as to yield the contents indicated, are very convenient. We are then never in doubt whether we have left too large a drop in the pipette; but we must always wait for a time, until the liquid flowing down from the sides has collected completely up to the lower mark.

§ 25. Now begins the titration. To the measured quantity of liquid in the beaker we allow the standard solution to flow in from the burette until the desired change has taken place. The following examples will show how this point is recognised:—

We can determine nitric acid quantitatively by the volumetric method if we allow solution of indigo to flow into the flask containing the nitric acid. We shall have added enough when the liquid begins to retain a blueish green colour, *i.e.*, when further quantities of indigo are no longer decomposed, as all the nitric acid is consumed. Here the standard solution itself serves as the indicator. From the number of *cc.* of indigo solution used it is easy to calculate the nitric acid.

If we wish to examine a pure, aqueous solution of sulphuric acid for its proportion of the latter, this may be effected most readily if we add

an aqueous soda-lye of known strength, until all the sulphuric acid is converted into sodium sulphate according to the equation

$$H_2SO_4 + 2NaOH = Na_2SO_4 + 2H_2O.$$

As the calculation shows :—

H_2	= 2	Na	= 23
S	= 32	O	= 16
O_4	= 4 × 16 = 64	H	= 1
	98		40

Ninety-eight grammes sulphuric acid combine with twice 40 sodium hydroxide ; if, therefore, we use a solution of 40 *grms.* sodium hydroxide in 1 litre (so-called normal soda solution, compare § 27), 1 *cc.* contains $= 0{\cdot}04$ *grm.* NaOH, and saturates $\frac{0{\cdot}098}{2}$ *grm.* $= 0{\cdot}049$ *grm.* H_2SO_4. We cannot here detect the point of saturation without an artifice, since soda-lye, sulphuric acid, and their product of conversion, sodium sulphate, are all alike colourless and soluble in water. But if we add a few drops of litmus as indicator to the sulphuric acid it takes a red colour, which changes to violet as soon as the last molecule of sulphuric acid is combined with the soda-lye, and into blue as soon as even the slightest excess of soda-lye is present. At the moment when the colour turns violet we cease adding soda, read off the quantity a consumed, and know that in our beaker there were present $a \times 0{\cdot}049$ *grm.* sulphuric acid.

These examples will suffice for an understanding of the principles of titration. It would take us too far, and would explain nothing further, if we were here to speak in general outlines of the various other chief methods of titration—the oxidimetric, the iodometric, &c. So much of these methods as is necessary for hygiene will be found in the second part.

§ 26. Appendix to § 25 : **The most important Indicators for Acidimetry.**

1. Tincture of Litmus.—The commercial blue cubes and balls of litmus consist of gypsum and chalk, saturated with the blue alkaline compound of the colouring matter of litmus, which is red in its free state. For preparing the tincture we first extract the mass with strong alcohol, which removes certain contaminating colouring matters, and which is then poured away. The residue is then digested with hot water, the filtrate is reddened by adding a few drops of sulphuric acid, and baryta-water is added until a strong blue coloration appears. The excess of baryta is precipitated by means of a current of carbonic acid, the excess of which is then expelled by boiling, and the liquid is filtered. The neutral violet solution is preserved in bottles closed merely with a tuft of cotton wool. In stoppered bottles there occurs reduction

involving decoloration in consequence of the growth of fungi. The artificial colouring matter, lacmoid, may also be used instead of litmus.

Litmus paper is in very frequent use, *i.e.*, slips of filter or letter-paper saturated with litmus tincture and dried. Violet, *i.e.*, neutral paper, is the best. The student must beware of deeply coloured red or blue paper, since it reacts less readily on account of an excess of acid or alkali.

Acids, even carbonic acid, redden litmus, as also do acid salts; alkalies give a blue colour. Litmus cannot be used for the titration of salts of boric acid and of magnesium.

2. **Turmeric Paper (Commercial).**—The yellow paper is not affected by acids, except the boric acid; traces of alkalies and of ammonia turn it brown. It is at the same time a very sensitive reagent for boric acid, which turns it to a brick-red. Compare § 227.

3. **Rosolic Acid (Coralline).**—A 1 per cent. solution in dilute alcohol of a yellowish red colour. Acids (even carbonic acid) and acid salts change rosolic acid to a yellowish or colourless liquid; alkalies turn it to a rose or a carmine red. Only a couple of drops of the reagent are added to the liquid which is being titrated.

4. **Phenolphthaleine.**—Three grammes dissolved in 100 *cc.* of dilute alcohol. Two drops of the indicator are added to 100 *cc.* of the liquid to be titrated. Traces of free caustic alkalies or alkaline earths give an intense violet red colour. Ammonia reacts only if in large excess. Acids (even carbonic acid) and acid salts leave the indicator colourless, or decolourise it if in excess.

5. **Congo Paper.**—Alkalies, neutral and acid salts do not alter the red colour, but free acids (especially the mineral acids and acetic acid) turn it blue.

6. **Poirrier's Orange III. = Dimethyl-aniline-diazo-benzol Sulphuric Acid.**—Generally known as *methyl orange.* Coloured yellow by alkalies and red by acids, though not by carbonic acid (and by oxalic acid only if in great excess). If a pure solution of alkaline carbonates is mixed with a mineral acid and rosolic acid, the colour turns yellow even on the addition of a slight quantity of mineral acid. This change is occasioned by the liberation of carbonic acid, which must be constantly expelled by boiling if an accurate conclusion is to be formed from the quantity of mineral acid consumed as to the quantity of carbonate. Carbonates may be decomposed in the cold with methyl orange as indicator, since the carbonic acid liberated does not act upon the yellow colour, which does not change to red until there is an excess of mineral acid.

§ 27. **Normal Solutions.**—Solutions of monovalent bodies which contain their atomic weight in grammes per litre in case of chemical elements, or, in chemical compounds, their molecular weight in the same volume, are called normal solutions; if they contain five times the quantity, or one-

tenth, &c., they are spoken of as five-fold or deci-normal solutions. Thus, *e.g.*, a normal solution of iodine contains 126·5 *grm.* iodine, a normal hydrochloric acid contains 36·4 hydrogen chloride per litre. If the normal solutions of polyvalent compounds have to be prepared, the molecular weight must be divided by the number of valencies.

Thus, *e.g.*, 1 litre of a normal solution of bivalent compounds contains:—

Sulphuric acid: $\frac{SO_4H_2}{2} = \frac{98}{2} = 49$ *grm.* H_2SO_4, or 40 *grm.* SO_3.

Oxalic acid: $\frac{C_2O_4H_2 + 2H_2O}{2} = \frac{126}{2} = 63$ *grm.*

Barium hydroxide: $\frac{Ba(OH)_2}{2} = \frac{170·9}{2} = 85·5$ *grm.* $Ba(OH)_2$ = 76·5 *grm.* BaO.

Calcium hydroxide: $\frac{Ca(OH)_2}{2} = \frac{74}{2} = 37$ *grm.* $Ca(OH)_2$ = 28 *grm.* CaO.

By this arrangement 1 *cc.* of any acid of whatever valency corresponds to 1 *cc.* of any alkali.

However, we do not always work with normal solutions, but often with liquids of purely empirical strength, as may appear most convenient for the time being—*e.g.*, instead of a solution of sodium chloride containing 58·5 *grm.* per litre, or an aliquot part of that number, we occasionally use a solution containing per litre: 1 *grm.* chlorine, 1 *grm.* sodium chloride, or 1 *grm.* hydrochloric acid, according to the purpose in view.

3. Colorimetric Methods.

§ 28. A number of substances, *e.g.*, iron, ammonia, nitrous acid, &c., have the property, if mixed with suitable reagents even in the minutest quantity, of producing intense colours; the strength of the colour is approximately proportional to the quantity of the substance.

Upon this reaction quantitative methods have been founded as follows: We procure a number of cylinders of colourless

glass, equal in width (about 2 to 3 *cm.*), having a mark at 100 *cc.* Into one of these we pour 100 *cc.* of the liquid to be examined, and into the others watery solutions of different strengths of the body to be determined. We add to the strata of liquid (which are of equal heights) equal quantities of the suitable reagent, and after stirring up we compare the colouring produced by looking down through the cylinders upon a white ground. If after several trials, and perhaps after diluting the liquid in question, we have obtained an equality of colour between it and the standard liquid, the strength sought for is known at once. If the operator has but little liquid at his disposal, the colorimetric analysis can, in case of need, be executed in test-tubes of equal width. A fixed time for the duration of the experiment is often necessary. For a complete instance see the determination of ammonia in drinking water.

We may recommend Hehner's cylinders, two exactly equal measuring cylinders with flat bottoms, and an outflow cock at the bottom, holding 105 *cc.* Into one of those cylinders are poured 100 *cc.* water, into the other 100 *cc.* of the liquid for comparison; equal quantities of the reagent are added to each. The contents of each are well mixed by stirring or shaking, and a portion of liquid is run out from the one with the more deeply coloured contents until an equality of colour appears on looking down from above. Of course the intensities of colour must not differ too widely before letting the liquid run out, otherwise the experiment will be inaccurate. The calculation is shown by the following example: 99 *cc.* of distilled water + 4 *cc.* of an iron solution, containing 1 *mgrm.* iron per *cc.*, have a deeper colour than 100 *cc.* of water which was obtained from 500 *cc.* spring water (by evaporation and treatment with potassium chlorate and hydrochloric acid), after there has been added to each ½ *cc.* of pure concentrated hydrochloric acid, and 1 *cc.* of solution of potassium ferrocyanide. When the comparison solution has been allowed to flow away down to 70 *cc.*, an equality of colour was recognised on looking through from above.

$\frac{4 \cdot 70}{105} = 2 \cdot 66$ *mgrm.* iron are present in the 70 *cc.* of the comparative solution, and consequently also in the 100 *cc.* of the water which has been concentrated by evaporation. But as they represent 500 *cc.* of the original water, 1 litre contained 5·3 *mgrm.* of iron.

More expensive colorimeters have hitherto met with scarcely any application in hygienic research.

Concerning quantitative spectrum analysis, which is essentially a colorimetric method, see note in § 15.

4. Quantitative Determination by means of Circular Polarisation.

§ 29. Many substances in solution have the property of turning the plane of a polarised ray of light, and this property has long been used for quantitative determinations. The simplest apparatus for rotatory polarisation are thus arranged (Mitscherlich): A sodium light polarised by a Nicol prism passes through a tube filled with the liquid to be examined, and then encounters a second Nicol prism. As long as the tube is empty, the darkness, on looking through the eye-piece Nicol, is most intense when the axes of the two Nicols cross each other. If the ray of polarised light is deflected by the liquid to the light, the eye-piece Nicol must be turned as much to the right until the maximum darkness is reached again. The magnitude of the deflection of the eye-piece Nicol is read off on a graduated scale.

"Mitscherlich's Half-Shade," constructed by Schmidt and Haensch, is much approved of as a cheap and at the same time relatively accurate instrument.

The observation is here effected by means of a small telescope with a vernier, which is not adjusted either for the greatest or the smallest brightness of the field of vision, but for equal brightness of both fields—that is, the field of vision appears divided vertically, a plate of quartz being introduced before the one lateral half of the polarising Nichol. A sodium flame is here also used as a source of light.

Larger Apparatus.—In the Wild instrument we observe how far the analyser must be displaced from its position of rest (45° towards the polariser) in order to see the disappearance of the interference bands, which appear in two peculiarly-ground quartz plates on the introduction of the tube filled with the fluid. In the Soleil Ventzke apparatus a quartz plate is inserted in the track of the polarised light deflected by the liquid, of such a thickness as to compensate for this deflection. Two wedges of quartz, which may be displaced by means of screws, enable the operator to produce a stratum of quartz of any thickness required.

The compensation is complete when the two fields of vision have the same tone of colour.

A more complete description and a theoretical explanation of these costly instruments is to be found in the more voluminous manuals of physics, and in the directions for use supplied along with the apparatus.

All the liquids to be examined with the polariscope must be clear, and have at most a faint colour. We generally work with liquids at 15°, using a tube of 200 *mm.* in length, which is to be exactly filled, without air-bubbles. If bodies are present which deflect the ray of light in the opposite direction (*e.g.*, albumen and glucose), the polarisation must be repeated after the removal or transformation of the one. (Compare Wine.)

The length of the tube is often so arranged (198·4 *mm.* instead of 200 or 220 *mm.*) that percentages of glucose (deflection to the right) or albumen (deflection to the left) may be read off at once. But it is decidedly preferable to possess an apparatus which allows of work with a tube of the normal length.

§ 30. The magnitude of the deflection a observed depends on—

$[a]$ = the specific or molecular rotation, *i.e.*, the deflection of a stratum of liquid 1 *dm.* in length, containing 100 *grm.* substance in 100 *grm.* of water.

l = the length of the tube in decimetres.

p = the percentage, *i.e.*, how many grammes of substance in 100 *grm.* water.

d = the specific gravity of the solution; and

c = the concentration, *i.e.*, how many grammes substance in 100 *cc.* of solution.

$$[a] = \frac{100\,a}{l\,p\,d} = \frac{100\,a}{l\,c} \text{ or } p = \frac{100\,a}{l\,d\,[a]};\ c = \frac{100\,a}{l\,[a]}$$

In general we state $[a_D]$, *i.e.*, the specific rotation for yellow light of the wave-length of the D line. For light of different wave-lengths, $[a]$ is often considerably different. Deflection to the right is indicated by the sign +, and that to the left by −.

In many substances $[\alpha_D]$ decreases for some time after the preparation of the solution (birotation). Thus, a solution of glucose does not reach its minimum of rotation until thirty hours after the preparation of the solution; it then remains constant. It is assumed, by way of explanation, that the groups of molecules which rotate more strongly are split up in the course of this time into molecules which rotate normally. It follows as a practical rule that all solutions prepared for comparison must be allowed to stand for about thirty hours. Compare Pribram, *Berichte der Deutsch. Chem. Gesellschaft*, xxi.

Table of the value of $[\alpha_D]$, at temperatures of 15° to 20°, for some of the more important substances in dilute solutions up to about 5 per cent:—

Cane sugar, $C_{12}H_{22}O_{11}$	+ 66·5
Milk sugar, $C_{12}H_{22}O_{11} + H_2O$	+ 54·0
Maltose, $C_{12}H_{22}O_{11} + H_2O$	+140·8
Fruit sugar (dextrose), $C_6H_{12}O_6$ . . .	+ 51·8
Fruit sugar (levulose) { at higher temperatures the deflection decreases greatly	−100
Invert sugar { at higher temperatures the deflection decreases greatly	− 28
Dextrine	+139 to 213
Serum albumen	− 56

For more complete tables see *Beilage zum Chemiker Kalendar*, Berlin, Springer, a new edition yearly. An excellent account of the entire subject, theoretical and practical, is found in Landolt, *Das Optische Drehungsvermögen*, &c., Brunswick, 1879.[1]

5. Measurement and Calculation.

(Supplementary to Chaps. I. to IV.)

§ 31. In scientific researches all magnitudes are stated according to the metric system, using the following abbreviations:—

Measures of Length.—1 metre (*m.*) = 10 decimetres (*dm.*) = 100 centimetres (*cm.*) = 1000 millimetres (*mm.*); 10 metres = 1 dekametre (*Dm.*); 100 metres = 1 hectometre (*hm.*); 1000 metres = 1 kilometre (*km.*); 10,000 metres = 1 myria-

[1] See also *Ganot's Physics*, translated by E. Atkinson. Eleventh edition. London: Longmans, 1883.—*Translator.*

metre (*Mm.*); 1 millimetre = 1000 micromillimetres, or 1000 mikrons (μ).

Superficial Measures.—1 square metre (*sm.* or □ *m.*) = 100 square decimetres (*sdm.*) = 10,000 square centimetres (*scm.*) = 1,000,000 square millimetres (*smm.*); 100 square metres = 1 are (*a.*); 100 ares = 1 hectare (*ha.*).

Solid Measures.—1 cubic metre (*cbm.*) = 1000 cubic decimetres (*cbdm.*) = 1000 litres (*l*) = 1,000,000 cubic centimetres (*cc.*) = 1,000,000,000 cubic millimetres (*cmm.*). Cubic centimetres in Germany are written *cbcm.* or *ccm.*, but in England and France *cc.*

The unit of weight is also derived from the metric system. 1 *cc.* water at 4° weighs 1 gramme (*grm.*) = 10 decigrammes (*dgrm.*) = 100 centigrammes (*cgrm.*) = 1000 milligrammes (*mgrm.*).

1000 *grm.* = 1 kilogramme (*kilo*).
500 *grm.* = 1 mi-kilo, or metric pound.
100 *kilos* = 1 metric cwt.
50 *kilos* = 100 metric pounds = 1 cwt.

The reduction of some old German and of some foreign weights and measures to the metric system is given in Table I.

§ 32. If lengths have to be measured very accurately we make use of a small apparatus called after its first inventor, Nonius, or Vernier, after its second. It consists of a small measuring-rule made to slide along a larger scale 9 *mm.* in length, but graduated not into 9 but into 10 parts. Each degree of the vernier is therefore smaller by $\frac{1}{10}$ *mm.* than a degree of the main rod (Fig. 16). To effect a measurement we proceed

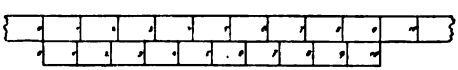


Fig. 16.—Nonius.

as follows: We apply as usual the measuring-rod to the object; only in the rarest cases the extremity of the object will coincide with one of

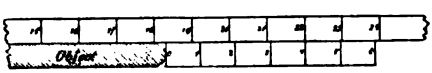


Fig. 17.—Nonius in use.

the graduation marks: in our example it falls between 18 and 19 (Fig. 17).

Hence our object is in length 18 degrees, and the fraction of a degree *a*. We push the lower end of the vernier close up to the object, and observe what degree of the vernier coincides with a degree of the main rule. In our example it is the fourth. Consequently $a = 0{\cdot}4$, since the four degrees of the vernier, 1 to 4, are exactly shorter than the four degrees of the main rule from 18 to 23 by four times 0·1 degree. We may thus easily measure with accuracy to $\frac{1}{10}$ *mm.*, even when the measure is divided only into entire millimetres. It most cases it is sufficient to read off the entire millimetres and estimate the tenths.

§ 33. Microscopic measurements of length are effected by means of so-called micrometers, *i.e.*, delicate measures marked upon glass, and graduated, *e.g.*, in twentieths of a millimetre. (Compare Behrens.)

Objective micrometers are marked upon the port-object, or the covering glass itself. Since they are magnified in the same proportion as the object, we can measure with them directly, without having recourse to any calculation. On the other hand, and for the same reason, they can be used only for coarser measurements.

For measuring very minute objects we use eye-piece micrometers. These consist of small glass slides which carry a delicate scale, and which can be introduced at a slit into the eye-piece of the microscope, between the ocular and the collecting lens. On looking into the microscope we then see the preparation or object strongly magnified by the lenses of the eye-piece and of the object-glasses, whilst the graduation of the micrometer is only slightly magnified by the eye-piece. In order to ascertain to what values a degree of the ocular micrometer corresponds, we require temporarily a second micrometer, which is used as an object. If we look into the microscope, *e.g.*, the 3 degrees of the objective micrometer (each of which we know corresponds, *e.g.*, to $\frac{1}{20}$ *mm.*) represent 16 degrees of the eye-piece micrometer. For the object-lens in use 1 degree of the eye-piece micrometer $= \frac{3}{20\ 16}$ *mm.*, *i.e.*, $= 0{\cdot}00937$ *mm.* $= 9{\cdot}37$ μ. In this manner the value of a degree of the eye-piece micrometer must be once for all ascertained and noted down for every combination of object-glasses.

§ 34. Concerning the measurement of macroscopic right lines there is nothing further to be said. Curved lines are often difficult to measure with exactness, which, however, is rarely required.

The circumference of a circle is found according to the formula $2\,r\,\pi$, where r is the radius or semi-diameter, $\pi = 3{\cdot}1416$, or, for approximate computations $= 3\frac{1}{7}$, or $\frac{22}{7}$.

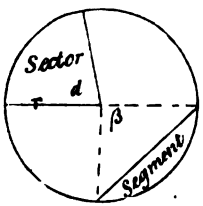


FIG. 18.

A portion of a circular arc (Fig. 18) is easily calculated if we know the angle (β) by which it is subtended. The measurement of angles is known.

$$b = \frac{2\,r\,\pi\,\beta}{360}.$$

§ 35. **Determination of Superficies.**—The extent of a regular superficies is found by a simple calculation after measuring the sides according to the following formulæ:—

Square $= s\,s = s^2$, s being the length of a side.

Rectangle $= g\,h$ where g = the base line and h the height.

Trapezium: $\left(\frac{g_1 + g_2}{2}\right) h$, where g_1 = one of the parallel sides, g_2 the other parallel side, h the height, *i.e.*, the perpendicular distance between the two parallels (Fig. 19).

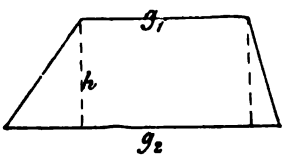


FIG. 19.

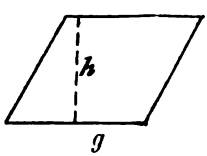


FIG. 20.

Parallelogram: $g\,h$, g being one side and h its vertical distance from the opposite parallel (Fig. 20).

Triangle: $\frac{g\,h}{2}$, where g is a side, h a perpendicular let fall from the angle opposite to this side to the side g or its prolongation (Fig. 21).

Irregular polygons are resolved into triangles by diagonals; each is then calculated separately, and the whole are added together. In regular polygons it is sufficient to resolve the figure from its centre into regular triangles, to calculate one of them, and to multiply by the number of sides.

Surface of a circle $= r^2 \pi$. r = radius; $\pi = 3{\cdot}1416$.

Sector (Fig. 18) $= \frac{r^2 \pi a}{180}$, where a is the central angle.

Segment (Fig. 18) $= \frac{r^2 \pi \beta}{360} - \frac{s h}{2}$, h being a perpendicular to the middle of the chord, and β the central angle.

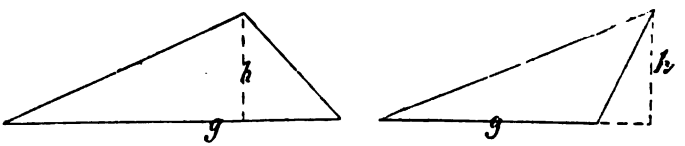


Fig. 21.

Irregularly-bounded plane surfaces are best determined by drawing them upon homogeneous paper, cutting the figure out and weighing, after having previously determined the weight of 1 *scm.* of the same kind of paper. A simple division then gives the size of the irregular superficies.

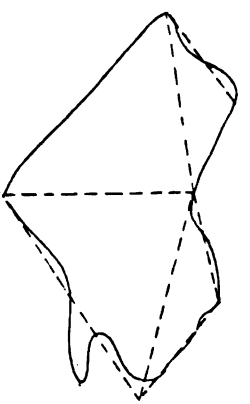


Fig. 22.

Large irregular surfaces which cannot be cut out (*e.g.*, the area of a garden, &c.) are calculated by substituting for them an irregular polygon, which approximates as closely as possible to their form (Fig. 22). The more accurate the calculation is to be, the more numerous angles must be assumed.

Of curved surfaces few require notice.

The surface of a round cylinder $= 2 r \pi h$, where r is the radius of the terminal end plane and h its height.

The surface of a vertical cone with a circular base =

$r\ \pi\ s$, where s is the shortest distance from the base and the vertex, measured on the mantle of the cone, and r is the radius of the base.

The superficies of a sphere $= 4\ r^2\ \pi$.

§ 36. **Determination of Cubic Space.**—The contents of the regular bodies which are met with in hygienic operations may be simply measured if we know the length of their sides.

Cube $= s^3$.

Prism (three-, four- [parallelopipedon], or many-sided, or with a round surface [cylinder]) always $= g\ h$, where g is the surface of the base and h is the height.

If the prism is oblique, h is put for the vertical distance of the two parallel surfaces.

Pyramids and Cones $= \frac{g\ h}{3}$, in which g is the surface of the base, h the perpendicular distance of the apex above it.

Sphere $= \frac{4}{3}\ r^3\ \pi$.

In all these cases it is indispensable that all the measures of length shall be expressed in one and the same scale—that is, all in metres, decimetres, centimetres, or millimetres. The result is then expressed correspondingly in square metres, square decimetres, square millimetres, or, as the case may be, in cubic metres, cubic centimetres, &c.

But if one magnitude is, for instance, measured in centimetres and the other in millimetres, on multiplying them an irrational result is obtained—a frequent error with beginners.

§ 37. The cubic contents of an irregular body (*e.g.*, a boulder or a beet-root) is calculated by weighing it, and dividing the number thus obtained by the specific gravity, *i.e.*, the weight of 1 *cc.* of the substance. But the specific gravity is mostly not sufficiently known, and to ascertain it its volume must be determined.

The volume of a body is found by suspending it from a fine wire, and allowing it to be immersed in a glass of water previously tared and set upon the pan of a balance. The increase of weight of the glass of water in grammes expresses

directly the volume of the body in cubic centimetres. For a more complete account of the operation see the section on "Soils."

In cases of small solids (*e.g.*, quartz stones), a burette is filled up to a certain mark with water, and the substances in question are dropped in. The increase of volume occasioned by the stones—in other words, the volume of the stones—can be read off on the graduation of the burette. If the substances are soluble in water (*e.g.*, crystals of alum), the burette is filled with oil; in other cases, petroleum, glycerine, and other bodies may do good service. Of course no bubbles of air must adhere to the small solid bodies.

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SECTION II.

BACTERIOLOGIC METHODICS.

§ 38. **Introduction.**—By schizomycetes, bacteria,[1] in the widest sense of the words, we understand simple, non-ramified, minute, and minutest organisms, of globular, rod-like, screw- or thread-like form, which multiply by transverse fission, and are devoid of chlorophyllic colouring matter. Except in some cases where flagella have been discovered, these beings are without differentiated organs.

Many kinds develop, under certain conditions, a shining permanent spore in the interior of the cell (endogenous formation of spores); in other kinds an arthrospore, a portion of the cell bound off, plays a similar part. Other methods of propagation are wanting.

The power of many kinds to produce pigments, fermentations, decompositions, and diseases renders their study of extreme hygienic importance, especially since the following methods have come into use, instead of simple microscopic examination:—

1. The observation of the dyed fungi.

2. The various methods of cultivation which permit both of enumeration and of the distinct colouration of the various species. By means of such cultivations, accurate experiments on the biological and pathogenic functions of individual kinds can be instituted.

1. Microscopic Search for Bacteria.

§ 39. For this purpose the first condition is an efficient microscope. With the medium microscopes formerly in

[1] An appendix to this section (§ 89, &c.) contains information on other low fungi (yeasts, moulds, &c.) of hygienic interest.

favour (*e.g.*, the admired Hartnack VII., with the eye-piece No. 2 or 3) we may distinguish large bacilli (splenic fever) satisfactorily; for minuter bacteria, especially micrococci, the bacilli of tuberculosis, &c., we require a $\frac{1}{12}$ homogeneous oil-immersion and an Abbe condenser, if accurate results are to be attained.

The signification of the oil-immersion is as follows:—

The loss of light by reflection at the boundary surfaces of mediums optically different is done away with, as the oil and the glass are equally refringent, and there is consequently produced an optically homogeneous stratum from the preparation or object to the first lens. At the same time a very considerable amount of spherical aberration is suppressed at its very origin. The anxious care for the thickness of the covering-glass is at an end, but a defined length of tube must be maintained for each immersion object-glass. In view of the far purer image which good homogeneous object-glasses yield in comparison with powerful dry combinations, the former allow of stronger eye-pieces, and therefore of a much more considerable magnifying power.

Quite recently Zeiss, and after his example other makers have produced so-called apochromatic objective combinations with accompanying compensation eye-pieces made of peculiar kinds of glass and calc-spar, and producing images of a degree of perfection not previously attained. The spherical and chromatic aberration is reduced to a minimum, and very powerful eye-pieces may come into use. These combinations are especially valuable for microphotography. Unfortunately such lenses are as yet very expensive.

§ 40. The oil-immersion is applied as follows:—A small drop of inspissated cedar oil, or a mixture of castor oil with a strongly refractive essential oil (*e.g.*, oil of fennel), is poured from a small bottle with a prolonged stopper, set apart for this purpose, upon the covering-glass of the preparation, which must of course be thin, not thicker than the first lens of the immersion-combination. The lens is then cautiously immersed into the oil by means of the coarse adjustment, or by hand, until an image is perceptible, and the focus is finally obtained by means of the fine adjustment. The management of the coarse adjustment is easily learnt by standing in a stooping position, and judging of the distance of the lens from the covering-glass.

The use of the suitable diaphragms, and of Abbe's condenser, is very important. The latter name denotes a com-

bination of lenses applied beneath the object-stand of the microscope. It throws a broad cone of light into the object, so that the structure of the image produced by differences in the refraction of light disappears, and only distinct, isolated, dark-coloured objects stand out in sharp, coloured relief.

Hence it follows that the condenser must be used only when coloured micro-organisms are under examination. If colourless bodies are examined they must be removed (lowered) by means of a sliding mechanism, or, as it is most usual, deprived of its action by the introduction of a very narrow diaphragm. The newly-invented iris-diaphragms (Fig. 23)

FIG. 23.—Iris-diaphragm.

allow of the production of any desired aperture by a pressure of the finger.

If the atmosphere is very transparent, a diaphragm of medium width will often be convenient, even in the examination of coloured microbia. If we wish to determine the relative positions of the bacteria and the cells, diaphragms of mean width are quite necessary, as the cells of the preparation, even when coloured in the ordinary manner, are made indistinct by the full action of the Abbe apparatus almost to disappearance.

In uncoloured preparations we must, on the contrary, beware of darkening the image too much by too narrow diaphragms in very powerful combinations or in dull weather. The most suitable diaphragms must be selected.

§ 41. The schizomycetes, although physiologically so distinct, possess only a limited range of forms, and as long as a system was exclusively built upon microscopic forms, without taking their development into consideration, a true foundation was wanting.

We must distinctly understand that two schizomycetes may be microscopically identical and yet may belong to totally distinct species, and that, on the other hand, there are species with very manifold types of growth.

Buchner has provided these forms of growth with names of German origin, so that they may not be made to serve as generic names. The following are his proposals, which have been almost universally adopted.

Simple Forms of Growth.

Globular form (*a*)—not coccus.

Oval form (*b*). Longitudinal measure at most double that of the transverse measurement.

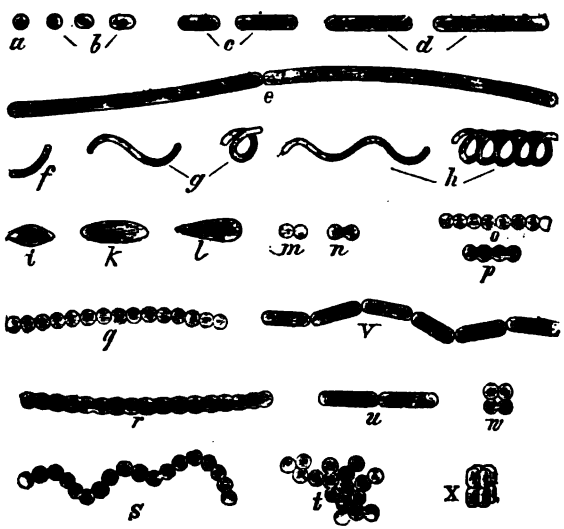

FIG. 24.—Forms of Bacteria according to Buchner.

Short rods (*c*). Length from two to four times the thickness.

Long rods (*d*). Length = two to four × thickness.

Filiciform (*e*).

Half-screw or comma (*f*), a very short section of a screw, not exceeding half a turn of the screw.

Short screw, a single short turn of a screw (*g*).

Long screw, a spiral (*h*). All screw forms may occur either with flat or upright threads.

Spindle (*i*).

Oval rod (*k*). Distinguished from a spindle by the ends being less taper, from the oval by its greater length = two to four diameters.

Club form (*l*).

Combined Growths.

Double sphere (*m*), separation only indicated.

Form of a roll (*semmel*) = biscuit form (*n*).

Series of globes (*o*) up to eight.

Torula form (*p*); the separation merely indicated.

Thread of globules (*q*); or of curved rosary form (*s*); if the separation is merely indicated by a torular thread (*r*).

Form of bunch of grapes (*t*); double rods (*u*); thread of links (*v*).

Lastly:

Tetrahedric form (*w*), a flat combination of 4, 8, 16, or more cells.

Cube (*x*), a combination of 8 to 32, &c., cells.

§ 42. The examination of undyed schizomycetes under a high magnifying power takes place relatively rarely, and chiefly in order to study the special movements of the organisms. For this purpose there is placed upon the middle of a covering-glass, *a*, *b*, by means of a platinum wire melted into a glass rod, or a platinum needle, a drop, *c*, of the bacteriferous liquid under examination, or a drop of broth infected with the cultivation to be examined. It is turned over, and laid with the drop downwards above the hollow of an object-slip, *A*, *B*, greased at the edge with vaseline, so that the drop may hang down free, hemispherical, and sharply defined into the small

concavity. If it is now examined with the narrowest diaphragm, we recognise the purely physical dancing molecular movement of the micrococci (the minutest lifeless particles

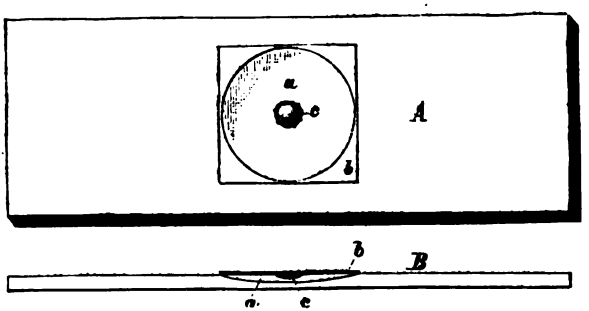

FIG. 25.—Examination in the Suspended Drop.

evince the same movement), the complete rest of the bacilli of splenic fever, the serpentine gliding of the bacilli of typhus, the darting of the vibrios of cholera, &c. For the staining of the flagelli, see § 47*a*.

The difficult adjustment for uncoloured organisms can be facilitated by first focussing with a low power on the margin of the drop, fixing the preparation with a clamp, and then examining with the oil-immersion.

For many bacteria we need an object-support which is capable of being heated, to see them in motion, e.g., *Bacillus prodigiosus*. It is best to heat the entire microscope by placing it during the observation in a heating-chest (L. Pfeiffer, *Zeitschrift für Hygiene*, vol. ii.).

Concerning the observation and culture of anaërobic bacteria in the suspended drop by means of pyrogallic acid and potassa-lye, see Nikiforoff, *Zeitschrift für Hygiene*, viii.

An uncoloured preparation may often do good service for recognising the presence of spores. The regular, generally oval (not globular) form, the strong lustre of (endogenous) spores, are in general easily recognised. Compare § 47.

The important investigations on the germination of spores, and other phenomena of growth, are likewise conducted in suspended drops of broth. See larger moist chambers in Hüppe. Of course, researches on the more minute morphological details must always be verified in uncoloured preparation.

§ 43. Where we are concerned with the simple and certain detection of bacteria in any substratum, staining[1] can now not be omitted. In case of liquids, or broad, moist masses in general, we proceed as follows:—

Upon the covering-glass, which has been cleansed and sterilised (flambé) by heating in a flame, we place a drop of the liquid in question, or some sterilised water in which has been stirred up a trace of the substance to be examined (as much as remained hanging on the point of the ignited platinum needle). Upon the first covering-glass we lay a second, and draw them apart, so that there remains on each only a very thin film of liquid. They are then allowed to dry. This is necessary for blood, sputum, pus, &c. If we wish to avoid distorting the blood corpuscles, the second glass is not superimposed, but applied obliquely, and moved in this manner over the lower glass.

In watery fluids the drop may be at once quietly allowed to dry on the covering-glass in the exsiccator, or the drying may be expedited by moving the covering-glass backwards and forwards at a height above the point of a flame.

Cultures, which have been developed in a suspended drop of broth, can be allowed to dry in a sulphuric acid exsiccator. In order to ascertain the more exact arrangement of the schizomycetes which have grown upon a solid nutrient medium, a covering-glass is laid cautiously and gently upon plate cultures (§ 65), lifted off with as great caution, and dried. (Flat preparation.)

When the bacteria have dried upon the glass, they must be fixed by a stronger heat. To this end the covering-glass is drawn three times through a Bunsen flame, with the coated side downwards (as rapidly as you would cut a slice of bread), which is about equivalent to a short heating to 115° to 125°. The preparation may then be stained.

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[1] On the theories of the staining of bacteria, whether chemical or physical processes play the leading part, particulars will be found in Hüppe. Unna has discussed this question thoroughly in the *Centralblatt für Bakteriologie*, vol. iii., Nos. 1 to 11. Both authors give the bibliography of the subject.

§ 44. We obtain our coloured liquids by first preparing saturated alcoholic solutions of aniline dyes (about 20 *grm.* colouring matter to 100 *cc.* alcohol at 90 per cent.), *i.e.*, methyl violet, gentiana violet, magenta, and methylene blue. These solutions are very permanent, and may be kept in stock for a long time. In general, magenta deserves the preference for staining; gentiana violet gives more permanent stains than methyl violet, but is apt to colour too deeply. Of these solutions we filter portions into ordinary medicine bottles, previously three-fourths filled with distilled water, using so much of the alcoholic solution of colour that the liquid in a stratum of 1 *cm.* in thickness (therefore as much as would reach to 5 *cc.* in a common pipette) begins to appear opaque.

FIG. 26.—

The antiseptic action of the aniline dyes permits of the use of water which has not been sterilised. The dilute solutions must be freshly prepared after two to three weeks, as granular turbidities separate out, which render neat work impossible. The bottles are then cleansed with crude hydrochloric acid, and the hands, if soiled with colouring matters, are cleansed with hydrochloric acid, crude or pure, diluted with an equal volume of water. The bottles are closed with corks which fit loosely, through which is passed a glass tube open at both ends and drawn out below (the simplest pipette). We close the glass tube with the finger, lift it up and allow a couple of drops of the coloured liquid to flow upon the covering glass. The bacteria are thoroughly stained in one to five minutes. The operation can be expedited by taking up the covering-glass with the forceps, and slightly heating the coloured solution cautiously over the flame. Still this is really always unnecessary. The excess of the coloured solution is then allowed to run off by inclining the covering-glass, which is next thoroughly rinsed with a plentiful stream of distilled water, when it may be at once examined in water. Or we dry the covering-glass after rinsing, which may be expedited by sucking off the water with blotting paper, and place it in a large drop of very liquid

Canada balsam[1] (Canada balsam dissolved in xylol) upon the port-object, when we have a very permanent preparation. Preparations which have first been examined in water may be afterwards dried and embedded in Canada balsam. If the preparation is to be doubly stained, we must use for the second preparation a weaker dye-liquid or a more prolonged rinsing in water.

Recently investigators who have many preparations to stain very frequently follow Neisser's method, and carry out the application of the bacteria and the staining upon the port-object, which is treated exactly as it has been described for the covering-glass. As port-objects are less easily broken than covering-glasses, and are more easily cleansed, this method has its advantages. In examining such preparations as are not to be preserved, we often content ourselves with putting upon the stained bacteria a drop of cedar oil, and examining them with the immersion lens without a covering-glass.

§ 45. For a large majority of schizomycetes the aqueous alcoholic colours prove sufficient; for those less easily dyed the colour must be mixed with a mordant, a substance which renders the schizomycetes more apt to take up colours. We may also strengthen the tinctorial action by allowing the colouring-glasses to float for hours upon a watch-glass full of colour, the coated side downwards.

The most important of these combined colouring-liquids are:—

1. Combination of methylene blue with potassa-lye.

Thirty cubic centimetres concentrated alcoholic solution of methylene blue; 100 *cc.* potassa-lye at 0·1 per thousand (0·1 *cc.* 10 per cent. potassa-lye + 100 water).

2. Combination of gentiana violet (or methyl-violet) with aniline-water.

Two cubic centimetres aniline oil and 20 to 30 *cc.* distilled water are vigorously shaken up in a small bottle or a test-

[1] Canada balsam is prepared by evaporating off about one-fourth of the commercial article on the water-bath, diluting the rest with xylol, and filtering.

glass. After some minutes the undissolved aniline oil is allowed to deposit, and the supernatant liquid is filtered through a filter previously moistened with water. To this clear aniline water there is added in a capsule so much concentrated alcoholic solution of gentiana or methyl violet that a faint indication of a film of colouring matter of a metallic lustre appears upon the surface. The aniline water must be freshly prepared each time, and the complete colouring solution keeps good only for a few days. It then becomes turbid, and dyes badly; but for a few days it may be preserved in a medicine-glass with a pipette stopper.

3. Combination of magenta with carbolic acid (Ziehl-Neelsen's colour).

Magenta	1·0
Absolute alcohol	10·0
Five per cent. carbolic acid . .	100·0

4. Compound of methylene blue with carbolic acid (Kühne's carbol-methylene blue).

Methylene blue, 1·5 *grm.*, and absolute alcohol, 10·0 *cc.*, are stirred up together, with the gradual addition of 100 *cc.* of a 5 per cent. carbolic acid.

Of these colouring solutions the first-mentioned (alkaline methylene blue) is rarely applied for staining preparations on covering-glasses, though it yields very fine results. Aniline gentiana and carbolic-magenta play an important part for covering-glass preparations, especially for staining the bacilli of tubercle, and for colouring spores. In both cases the objects do not take up the ordinary dye solutions, but they become dyed energetically in those containing mordants, and they do not readily abandon the colour which they have taken up even in contact with powerful decolorising agents. Carbolic methylene blue is especially recommended for the bacillus of glanders.

§ 46. **Bacilli of Tubercle** (coloration according to Ehrlich-Koch).—The preparations on glass covers, treated as usual, are allowed to float for twelve hours upon aniline gentiana. Others allow them to float only for five to ten minutes upon

a solution of aniline gentiana, previously heated to boiling. The simplest method is to hold the coloured liquid upon the covering-glass for some minutes over a Bunsen burner, in gentle steam, without allowing it actually to boil. It is then diffused for a short time (at most one to four seconds) in nitric acid at 30 per cent., and it is then rinsed, still more briefly (a few moments), in alcohol at 60 per cent. Other authors use, instead of the two liquids together, alcohol alone, mixed with 3 per cent. nitric acid, and finally immerse for a few minutes in a watery solution of Bismarck brown and rinse with water. In the acid and the alcohol used afterwards everything is decolorised (schizomycetes and tissues) except the bacilli of tubercle. A subsequent staining gives the decolorised parts a brown tone, whilst the violet of the bacilli of tubercle remains. The double dyeing shows the bacilli of tubercle more distinctly.

Instead of combining aniline gentiana and Bismarck brown, we may use in succession aniline magenta, and aqueous methylene blue. In this case the bacilli of tubercle appear red upon a blue ground.

Of the endless modifications of the method of staining tubercle bacilli one only may be mentioned, that of Ziehl-Neelsen, which is often used, and which Hueppe particularly recommends.

He dyes with carbolic magenta for ten minutes in the cold (or for two to three minutes at a gentle heat), places the preparation for a few seconds in sulphuric acid at 25 per cent., then for a few seconds in alcohol, rinses thoroughly with water, and dyes a second time with a concentrated aqueous solution of methylene blue.

Gabbet's modification, which is at present much approved, combines, as B. Fränkel first proposed, decoloration and subsequent redyeing by plunging the preparation, which has first been dyed with carbolic magenta, into sulphuric acid at 25 per cent., which has been coloured deeply blue with methylene blue. It is then rinsed with water. The use as a decolorising medium of alcohol containing only 10 per cent. of sulphuric acid, or 1 per cent. of hydrochloric acid, seems

to me less violent. Czaplewski gives a critical discussion of the entire literature of the subject: *Die Untersuchung des Auswurfs auf Tuberkelbacillen*, Jena, 1891.

For the demonstration of scattered bacilli in thin liquids (*e.g.*, urine), we examine the sediment deposited on standing quietly in the cold or extracted by centrifugal action; denser masses (sputum) are first rendered more fluid by dilution, and are then allowed to deposit. For this purpose there exist two methods complementary to each other.

1. According to Biedert: 15 *cc.* of sputum are well mixed up by stirring with 30 *cc.* water, four to eight drops of solution of caustic soda are added, and the mass is boiled with the gradual addition of 70 *cc.* of water until it becomes a thin liquid. For twenty-four to forty-eight hours the mass is allowed to deposit in a conical glass. The liquid is poured away and the sediment is examined. In order to cause it to adhere better to the glass cover, there may be added a little egg albumen or a little of the untreated sputum. This method yields quick and good results, but single bacilli lose their property of being dyed.

2. According to Strohschein: To 5 *cc.* of sputum there are added in a stoppered bottle 10 to 15 *cc.* of water, a knife point full of borax, and the same quantity of boracic acid. The whole is vigorously shaken up for a minute. After four to five days the liquefaction is effected. The sediment seems to preserve the tubercle bacilli very completely in a state capable of being dyed, and even after the lapse of two years, as I have convinced myself. The sediments are aseptic, and the bacilli admit of being dyed.

The bacilli of leprosy, which morphologically much resemble tubercle bacilli when dyed, like the latter are equally resistant to acids, or, according to some authors, more resistant. The chief difference is that the bacilli of leprosy dye well in cold aqueous magenta in six to seven minutes, whilst tubercle bacilli do not. On the other hand, leprosy bacilli do not dye in alkaline methylene blue (Löffler's) so well as do tubercle bacilli.

Bacilli of Syphilis and Smegma.—The organisms morphologically similar to the bacilli of tubercle which are found in the interior of recent syphilitic growths, and, on the other hand, in the *smegma præputii et clitoridis*, when stained with carbolic magenta, behave rather

differently than do the tubercle bacilli in contact with decolorising agents. On this method of staining, lepra behaves exactly as tuberculosis.

1. Alcohol applied directly decolorises the bacilli of smegma, but not those of syphilis and tuberculosis.

2. Mineral acids on direct action discharge syphilis at once, smegma more slowly, tuberculosis most slowly.

3. After the action of mineral acids until the bacilli of syphilis are decolorised, alcohol discharges smegma bacilli at once, but those of tuberculosis retain their colour.

§ 47. Gram's method (compare § 51), which stains the bacteria alone, gives very good results with covering-glass preparations, and is especially advantageous when a differential diagnosis is required.

There remain coloured on Gram's process:—

Micrococcus of the trachoma (Michel).

Micrococcus tetragonus.

Diplococcus of pneumonia (Fränkel).

Staphyloccus pyogenes aureus, albus, &c.

Streptococcus pyogenes and rysipelatis.

Bacillus anthracis.

Bacillus of the septicæmia of mice and of swine dysentery.

Bacillus of tuberculosis (little suitable).

There are decolorised:—

Diplococcus intracellularis meningitidis.

Diplococcus of gonorrhœa.

Bacillus typhi abdominalis.

Bacillus mallei.

Bacillus œdematis maligni.

Bacillus cuniculicida or choleræ gallinarum.

Bacillus of pneumonia (Friedländer).

Bacillus coli communis.

Bacillus neapolitanus.

Bacillus of the intestinal diphtheria of rabbits (Ribbert).

Bacillus mallei.

Spirillum choleræ and its nearest allies.

In the examination of blood great care must be taken lest cellular granules, which often occur abundantly in the leucocytes, and in part are stained exactly like schizomycetes, be interpreted as micrococci. In the former, indeed, we fail to find the equable roundness and the size of the micrococci. The granules are always enclosed in cells, and never lie free in the plasma, so that it can possibly be mistaken for blood only by hasty observers.

Günther has recommended the following method of avoiding confusion with the granules:—

The cover-glass preparations, after being passed through a flame, are

laid for ten seconds in acetic acid at 1 to 5 per cent., the acid is washed thoroughly away, the preparations are dried and stained in the usual manner. Only the bacteria take up the colour.

§ 47*a*. All the movements of bacteria are occasioned by flagella. Löffler (compare *C. f. B.* vi. 209, and vii. 625) has shown how they may be dyed with certainty. It is necessary to follow his directions exactly. We prepare:—

1. *Liquid mordant* mixed up of:—

10 *cc.* of a strong solution of tannin (20 *grm.* tannin in 80 *grm.* water).

5 *cc.* solution of ferrous sulphate saturated in the cold.

1 *cc.* alcoholic solution of magenta.

2. *Dye-liquor*:—

100 *cc.* aniline water (see § 45) saturated with solid magenta.

Add carefully a 1 per cent. solution of soda-lye until a stratum of several centimetres in depth is on the point of becoming opaque.

To the 16 *cc.* of the liquid mordant we add 1 per cent. of soda-lye, or an equivalent quantity of acetic acid in drops (22 drops = 1 *cc.*), according to the kind of bacteria.

The addition of alkali is necessary for—

Micrococcus agilis	19–20
Typhus	21–22
Charbon symptomatique	36

An addition of acid is required for—

Spirillum concentricum	1
Cholera	2
Proteus	2
Vibrio Metschnikoff	3–4
Bacillus pyocyaneus	5–6
Spirillum rubrum	6
Bacillus cyanogenus	10

For many fungi it is absolutely necessary to hit the exact degree of alkalinity. *Bacillus cyanogenus* is very slightly sensitive, and is therefore well suited for practice. The solutions are used only when quite fresh, their brisk mobility having been tested in the hanging drop. A very little of the

culture is stirred up in some distilled water; a small drop is then spread very thinly upon a covering-glass, which has been carefully cleansed successively with concentrated sulphuric acid, water, ammonia, and alcohol; it is then passed three times through a flame, avoiding too strong a heat. The glass is covered with the mordant, heated for one-half to one minute until steam begins to be formed, and rinsed first with water and then with absolute alcohol. A drop of the dye-liquid is then placed upon the glass; it is again heated for a minute, until steam is formed, and rinsed off with water. The form and the number of the flagella is very varied in different species, and their length is often considerable. *Micrococcus agilis* has several long flagella; among the movable bacilli *B. pyocyaneus* has only one flagellum, but the majority have several, or many which are sometimes confined more to the ends, but often (typhus) originate from the entire upper surface of the bacillus.

§ 48. The formation of permanent oval spores, refracting light strongly, was observed first by Cohn in the hay bacillus, and by Koch in the bacillus of splenic fever. For a considerable time the property of forming spores was regarded as an exclusive property of the bacilli. Recently the entire question which schizomycetes form spores has reached a stage which does not permit of a certain decision. Hauser detected endogenous spores in a *sarcina*, and Ernst in *sarcinæ* and in a *micrococcus*. The latter author discovered reactions which seem to indicate a preliminary stage of the spores. In some schizomycetes regarded as typical bacilli the demonstration of the spores has not been effected. (Typhus.)

Hüppe, who first maintained the abstriction of globules in the cholera vibrios as arthrospores, now views the larger cells in the series of streptococci as arthrospores, and very recently he even distinguishes arthrosporous and endosporous streptococci. (See Neisser, *Zeitschrift für Hygien*, vol. iv. p. 192.)

In the present work, which subserves practical objects, we

shall speak only of spores when structures have been demonstrated which are not only morphologically distinct (oval, shining, regularly arranged), but are also endowed with a higher power of resistance. Thereby the arthrospores are for the present eliminated from the circle of our consideration.

All the genuine endogenous spores capable of physiological resistance behave towards colouring-matters very like the tubercle bacilli—they take up colours with great difficulty, and retain them as obstinately. The spores of *Spirillum rubrum* form an exception. If we treat a thread of the bacillus of splenic fever, which is beset with spores, in the manner given for dyeing bacteria, the spores form undyed gaps in the dyed filament.[1] The spores may be dyed in the following manner:—

Double Staining, according to Neisser.—Cover-glass preparations which have been got ready in the ordinary manner are placed in a capsule with aniline methyl violet (or aniline magenta), which is heated in the water-bath or over a very small flame. The bacilli are decolorised by treating the preparation with absolute alcohol, to which there is added a little of the colouring matter which has been used. Or if this is not sufficient, the preparation is first plunged for a moment in 10 per cent. nitric or sulphuric acid. From the alcohol the cover-glass is transferred into a dilute aqueous complementary colour—methylene blue if magenta has been used, or methyl violet if Bismarck brown. Carbolic magenta may also be used for staining spores.

Hauser has recommended a very simple procedure: The cover-glass preparation is first passed ten times through the flame when dry (instead of three times), then grasped with the forceps and touched with the ordinary alcoholic-aqueous solution of magenta, then taken thirty to forty times through the Bunsen flame, carefully supplying what is lost by evaporation so that the liquid is always close on ebullition. The dyed preparation is decolorised for a short time (ten to

[1] We must, however, beware of regarding all such gaps as spores.

thirty seconds) with 25 per cent. sulphuric acid, well rinsed with water, and re-dyed in dilute methylene blue. This method seems to me especially worthy of recommendation.

Buchner heats the coated cover-glasses from thirty to sixty minutes in the drying-closet to 210°, or touches them with concentrated sulphuric acid, which is rinsed off after fifteen seconds. In such preparations the spores only are dyed, not the bacteria.

The preliminary stages of the spores (small, shining granules) dye like the schizomycetic protoplasm. Many observations are made on the developed spores; the property of taking up colours differs much in the various species, and at various stages of development.

Arthrospores, according to Ernst and Hueppe, come up well by dyeing the ordinary cover-glass preparations in alkaline methylene blue (for a short time in heat, but for a longer time in the cold), rinsing with water and re-dyeing with Bismarck brown, when they appear blue among the brown vegetative cells. The same method, according to Ernst and Babes, in many bacteria renders visible the granules which appear previous to the spores. These granules can be dyed with grain-black and hæmatoxyline, and according to Ernst may be regarded as nuclei. (Ernst, *Zeitschrift für Hygien*, vol. v.; Babes, *ibid.*)

§ 49. Bacteria suspended in liquids are more easily stained than those which have penetrated into animal or vegetable tissues, because, if no expedients are used, the ordinary colours for bacteria dye the tissue also strongly. The following process is to be recommended if animal tissue has to be microscopically examined for bacteria:—

1. *Smear Preparations.*—A fresh surface is laid open with a sterilised knife or scissors; the surface is touched with a platinum wire, the point of which is bent into a loop, and the traces of blood and organic juice which are thus obtained are applied on the cover-glass, which is then dyed as above directed.

Sausage, cheese, vegetable tissue, &c., are to be examined

in the same manner, and if they are deficient in water they must first be moistened with sterilised water.

Von Sehlen recommends, for fixing upon the glass dry materials which are to be examined for bacteria, a mixture of equal parts of boracic acid at 4 per cent. and fresh egg-albumen. The mixture, after being well shaken, is filtered, and applied on the glass in a thin layer. The fixation of the dust to be examined is effected by heating for a very short time to 100°.

Though in this manner we may obtain evidence of the presence of bacteria, the method affords no certainty as to their position. For this purpose we require:—

2. *Section Preparations.*—The animal organs on dissection, immediately after removal from the body, are placed, in pieces of the size of a bean or a hazel nut, in absolute alcohol,[1] which is charged once after twenty-four and forty-eight hours. After twenty-four hours longer, small portions of liver, kidney, spleen, heart, muscle, and other of the more solid organs,[2] are fit to be cut into sections, and, when superficially dried, can be fixed upon a cork by means of glycerine gelatine.[3] This is effected in the simplest possible manner by melting the glycerine gelatine on the water-bath (or very cautiously over an open flame); a large drop is taken up by means of a glass rod, and laid upon a roughened cork,

[1] Sections (Catheart microtome) of fresh organic matter which have been frozen by means of ether spray are to be recommended. It is still better to allow the portions of the organism which have been hardened by steeping in alcohol for several hours to freeze in water. The sections, which have been cut with a dry knife, are placed in a ½ per cent. solution of sodium chloride, and carefully transferred upon a spatula into alcohol, first at 60, then at 90, and finally at 100 per cent. This method does not seem to me to possess an advantage over that of adherent preparations, nicht "*Ausstrichprap.*," sondern "*aufgeklebtes Praepar.*"

[2] In the glass of alcohol there is placed, at half its height, a diaphragm of blotting-paper. The aqueous alcohol sinks down, so that the organs always lie in stronger alcohol. The organs of small animals, such as mice, are wrapped up together in a piece of muslin, to which is fixed a paper label *written with pencil;* a thread is passed through larger portions of organs, to which the label is secured. In this manner the organs of different animals may be preserved in one and the same glass.

[3] One part gelatine, seven parts of glycerine, six parts of water, are melted together in the water-bath: 1 *grm.* phenol is added to 100 *cc.* of the mixture, and the whole is filtered.

and the portion of the organism is then pressed with its base into the gelatine. By rapidly applying a little more of the gelatine jelly around the lines of contact, the specimen can be cemented more securely. The adhering preparation is then returned to the alcohol, which hardens it perfectly in six to twelve hours. The cork is now fixed in a screw clamp, and by means of a sliding microtome, any desired number of slides can be cut of a suitable thickness—0·01 to 0·03 *mm.* The sections are cut with a knife well moistened in alcohol, and fixed very obliquely. They are then removed with a clean brush into a capsule of alcohol, in which they may be kept for days if well closed up. If the cover of the capsule does not fit perfectly, fresh alcohol must be frequently added, since as the alcohol evaporates saprophytic fungi may be developed in the remaining water, especially if weak alcohol has been used.

A store of sections may be preserved in small stoppered tubes with alcohol.

If the experimentalist has no microtome at his disposal, he may, with a little practice, learn to cut serviceable sections by hand with a sharp razor.

In the exceptional cases where cementing does not suffice (for obtaining very delicate sections of the mucous membrane of the bowel, or through non-hepatised lung, &c.), the dehydrated portions of tissue have to be imbedded in celloidine or paraffine. Imbedding is also used for series of sections with the transverse knife.

Celloidine.—We prepare a thin and a syrupy solution of fine cut celloidine (celluloid) in a mixture of equal parts of alcohol and ether. The small pieces of tissue, perfectly dehydrated in absolute alcohol, are placed for some hours in a mixture of alcohol and ether, then for some time in the thin, or afterwards for twenty-four hours in the thick, solution of celluloid. A round cork is loaded below with lead, surrounded with a projecting margin of paper, some of the thick solution of celluloid and the piece of tissue are then placed in the tray thus formed, and the cork is sunk in a vertical position in alcohol at 80 per cent. The celluloid soon hardens, the knife for cutting is kept moist with alcohol at 80 per cent., the sections are laid in alcohol of the same strength, the celluloid is extracted in absolute alcohol, and the sections are dyed.

Paraffine.—The tissues, thoroughly dehydrated in absolute alcohol, which is repeatedly changed, are placed for twenty-four hours in xylol, and then in melted paraffine, which is kept at 45° to 55° in a regulated, heated apparatus. For working in hot weather sparingly fusible paraffine is taken, and inversely. The sections are cut with a dry knife and placed for six to eight hours in xylol, which is twice changed, and then twice in fresh absolute alcohol. The staining is begun when all the paraffine and the xylol are extracted.

We may, however, as is often preferable, dye the sections when they are saturated with paraffine and thus rendered solid, and quite at last when the tissue and the bacteria are both coloured, extract the paraffine with xylol.

§ 50. In unstained sections, as we may easily convince ourselves, we recognise bacteria only when they lie together in great heaps (balls of micrococci); we must consequently render them visible by means of suitable staining.

In order to stain sections in alcohol, we spread out, in the first place, one upon a nickel-silver or glass spatula, by means of a needle, lift it up out of the alcohol, the excess of which is removed with slips of filter-paper. It is then immersed in alcohol whilst still lying upon the spatula, from which it is then detached in order to make room for a second section. If in a few minutes the staining is sufficient the section is laid upon the spatula, lifted out, the excess of colour is removed, and the section is placed in the next liquid, &c. It is never well to take too many sections in hand at once, especially if we work with opaque, highly concentrated colours, such as aniline methyl violet or the like. Very thin sections are left upon the spatula during the entire process of dyeing. On transference from water to alcohol the sections are apt to shrink and double up, but on removal from absolute alcohol to water sections, even if much shrivelled, generally open well out. Thin sections are thereby often liable to tear.

The oldest method of staining bacteria, that of Weigert and Koch, is still very well applicable in many cases. The sections are placed in a capsule with an alcoholic solution of magenta or methyl violet diluted with water, as already described. After about five to ten minutes they are transferred to water, or better, to water containing two drops of acetic acid to each

10 *cc.* The loosely adhesive excess of colouring matter is thus rinsed off, and is farther extracted by means of water (which is changed, if needful) from the cellular mass, the nuclei and the bacteria alone remaining dyed. Water alone generally gives images which are less perfectly differentiated. No absolutely fixed rule can be laid down for the time of action of the dilute acetic acid, two to three minutes being a mean duration. If the sections are now examined in water we have in general a satisfactory image, but not rarely on attempting to place the sections successively in alcohol, absolute alcohol, xylol, or xylol Canada balsam, so as to obtain a permanent preparation, the result becomes considerably worse, as the alcohol extracts fresh quantities of colouring matter from the cellular nuclei and the bacteria. The decolourising power of the alcohol may be reduced by adding to it a little of the colouring matter which it is not to remove. If the preparation has been very intensely stained in the first operation, we may use for decolourising, instead of water, alcohol or alcohol acidulated with a little acetic acid; the last agent acts very strongly, often too strongly.

Carbolic magenta dyes better than aqueous magenta; for its removal we use, according to Koch, a solution of potassium carbonate (concentrated solution + water). The solution is allowed to act for five minutes, and is followed by alcohol containing a little magenta, pure absolute alcohol (for a moment), xylol, Canada balsam.

Löffler's alkaline methylene blue often yields very fine images. By this method the greatest number of bacteria can be successfully demonstrated. After the section has been steeped for some minutes in the colour it is placed for a few seconds in acetic acid at 1 per cent., and then successively in absolute alcohol, xylol, and balsam. If, according to Löffler, we add to the acetic acid a little tropeoline until it takes the colour of Rhenish wine, the protoplasm is stained yellow, whilst the nuclei and the bacteria appear blue.

§ 51. The finest method of staining bacteria in the tissue is that of Gram. It does not stain the cells and cell-nuclei,

but merely the bacteria, though unfortunately not all of them.

He transfers the sections (or the cover-glass preparations as fixed by heat) from strong alcohol into a capsule of aniline gentiana solution. When they have taken an intense blue-black colour (twenty minutes), they are placed for a short time (three minutes) in a solution of iodised potassium iodide (1 *grm.* iodine, 2 *grm.* potassium iodide, 300 *grm.* water), which causes copious precipitates of colouring matter. If the preparation, which has a dirty brownish green colour, is now transferred to absolute alcohol, it becomes of a blueish red, and the precipitated colouring matters dissolve with a purple colour. On adding continually fresh portions of alcohol, the preparation becomes by degrees quite colourless. If this is very difficult, the section may be again once more plunged for a short time into the potassium iodide solution.

If the aniline gentiana is allowed to act for a short time only, *e.g.*, half to one minute, the decoloration takes place much more rapidly, but the bacteria, though well stained, easily lose their colours on keeping.

Weigert has improved Gram's method as follows: He transfers the sections from the iodine solution, after they have been pressed between four folds of blotting-paper, at once to a mixture of two parts aniline and one part xylol, in which the differentiation and dehydration take place (this solution must be changed two or three times), then to pure xylol, and, lastly, to Canada balsam. According to this modification the fibrine, whose filaments have been sometimes regarded as fungoid filaments, retains a pure blue colour, whilst the schizomycetes become of a blue-black.

If we have to examine a tissue for bacteria the tinctorial properties of which are not known, it is best to apply the three methods described successively, each on two or three sections. The most universal method is that of Löffler, staining with alkaline methylene blue.

The bacilli of tubercle are dyed in tissues exactly as upon glass-cover preparations (§ 46), though it is here better to allow the cold solution to act for a longer time rather than

to apply heat. Fine images may be obtained both with aniline gentiana and carbolic magenta. Instead of sulphuric acid at 25 per cent. we use acidified alcohol. The double staining is effected as in § 51*a*.

The bacilli of leprosy are also stained in the same manner; but, as a distinction from tubercle bacilli, they are stained also in aqueous aniline colours. Alkaline methylene blue dyes the bacilli of tubercle satisfactorily in thirty minutes, whilst the bacilli of leprosy are but imperfectly dyed in one or two hours.

Spores rarely occur in bacteria imbedded in the tissues. The bacilli of tubercle form perhaps an exception; the staining of spores in the tissues has hitherto not been effected.

§ 51*a*. **Double Staining.**—If it is desired to have the bacteria stained with a colour contrasting with that of the tissues, the bacteria are preferably dyed violet, and the tissues are previously dyed with carmine, according to the histological methods of staining.

I should chiefly recommend to stain the tissues first with borax carmine, for which we require two solutions:—

1. Carmine	0·5	2. Hydrochloric acid	1·0
Borax	2·0	Alcohol	70·0
Water	100.0	Water	30·0

Mixture 1 is heated to ebullition in a porcelain capsule; dilute acetic acid is added by drops, whilst stirring, until the blueish red colour changes to a carmine red.

After standing for twenty-four hours it is filtered, and mixed with a few drops of liquified phenol (pure).

Sections are stained diffusedly in this solution in a few minutes. If they are then placed in solution 2, the differentiation is effected in a few minutes; colouring matter is given off in abundance, and only the nuclei remain stained. Such a preparation is well suited for treatment by Gram's method for staining the bacilli of tubercle, &c.

If, instead of staining the tissues first, we wish to do this

afterwards, it is best, after having dyed the bacteria violet, to use Bismarck brown; or, if they have been stained red, to apply dilute methylene blue.

2. The Preparation of Nutrient Media. Apparatus for Incubation.

§ 52. Equally important with the demonstration of the schizomycetes by staining is their recognition by culture. Many schizomycetes, *e.g.*, numerous cocci, cannot be distinguished from one another by the microscope; when recent they are colourless, and when dyed they appear as blue or red spherules, whilst their cultures often present respectively quite different figures.

In certain cases it may be almost impossible to detect in sections bacilli which are very thinly scattered in the tissues, whilst it is easily practicable by cultivation. Lastly, we know many organisms which are common in soils, dust, water, &c., but which exist so isolated that neither microscopic examination nor cultural experiments on ordinary media promise much result, whilst the organisms in question multiply abundantly if we embody them in an animal, *e.g.*, the bacilli of tubercle, the septicæmia of rabbits, &c.

We conduct our cultures upon solid or liquid media, absolutely sterile, and protected against contamination from without. Solid, transparent substrata play the greatest part in bacteriology, those especially which can be rapidly changed from a solid to a liquid state, or the reverse.

§ 53. For effecting sterilisation, which must always be preceded by a thorough cleansing, we proceed as follows:—

Metallic objects and small articles of glass can be sterilised by direct ignition in the flame of a Bunsen burner (platinum wires, scissors, knives, forceps, glass rods). A red heat is not necessary, and sterilisation is effected in half a minute. But even such a brief ignition suffices to destroy the sharpness of the instruments.

Glass apparatus (as also metallic objects, if the necessary

time is available) are heated in the drying-closet, a rectangular chest made of sheet iron or copper, put together with hard solder, and having double walls. A double or threefold burner quickly raises a thermometer passing into the interior to 150° to 160°. When this temperature has been maintained for thirty to forty minutes, the most resistant spores are destroyed.

As a matter of course the glass vessels have to be previously most thoroughly rinsed, and finally cleansed with distilled water. New glass often gives off alkalies to nutrient media which are boiled in it. A steeping for twenty-four hours in dilute hydrochloric acid (on which must, of course, follow a thorough washing with water) removes this objection. Thin-sided glass vessels, test-tubes, flasks, &c., may be placed in the cold drying-closet whilst still moist from the last drops of water not drained off, heat being then applied afterwards. Vessels of thick glass occasionally crack; this may be avoided to some extent if we sterilise at first with the door of the drying-closet half often. At present, however, dry sterilisation is not so much practised as formerly, since it has been found that test-glasses, flasks, &c., in which it is intended afterwards to preserve nutrient media, which have afterwards to be sterilised in steam, do not require dry sterilisation.

The plugs of wadding made of purified cotton wool firmly pressed together, and used as microbe-proof stoppers for the necks of culture-flasks, test-glasses, &c., can be sterilised in this manner; they turn slightly brown, but remain fit for use. It is the same with paper, linen, &c.

On the other hand, a dry heat is inapplicable when articles of caoutchouc, stoppers and tubes, are to be sterilised. This is best effected by exposure for thirty minutes to a current of steam or plunging into boiling water for the same length of time. Of course these articles are to be thoroughly dried with sterilised paper, and to be preserved wrapped up in sterilised paper, and not for too long a time.

For articles which cannot without injury be exposed either to a strong dry heat or to the action of sublimate or other

disinfectants (especially nutrient media), we have recourse to hot steam, which is effected conveniently and economically in Koch's steam-pot.

A cylinder of sheet iron or copper 75 *cm.* in height and 30 *cm.* in width, and coated externally with felt, is filled with water to about a fifth of its height. At about one-third of its height is a sheet-metal grating upon which the articles to be sterilised—liquids, gelatines, potatoes, &c., are placed in suitable vessels closed with stoppers of wadding. Upon the cylinder is placed a flat conical vessel, also coated with felt, and laid on loosely (not tight). Through a hole in its apex passes a thermometer, which extends down into the steam-chamber. If two or three burners are placed below the cylinder the water soon begins to boil, and the thermometer shows the boiling-point of the locality, *i.e.*, 100° at the sea-coast and in low plains, and 98° to 99° in hilly countries. After this point has been reached half an hour generally suffices (see § 53) for sterilising the nutrient media which have been introduced. These simple directions must be strictly observed in order to obtain a current of steam in a manner fit for use. The apparatus is very commonly left in action for one hour.

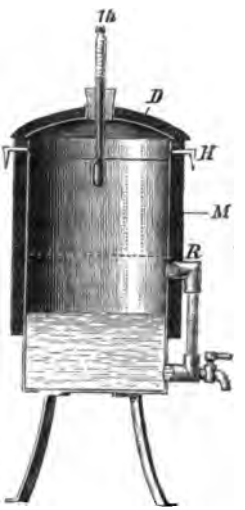

FIG. 27.—Koch's Steam-pot.

Stoppers of cork or of caoutchouc, paper, linen, vessels, &c., may also be sterilised in this manner.[1] In default of gas the apparatus may be placed upon a kitchen fire.

More expeditious and energetic than Koch's simple steam-pot, which works without pressure, are the so-called autoclaves, solid metal cylinders with covers capable of being screwed on and provided with an adjustable safety valve. These instruments effect sterilisation at 100° and 120° under the pressure of several atmospheres. In order to effect certain sterilisation it is simply requisite to leave the cock open for two

[1] On sterilisation by filtration, see § 79.

or three minutes after the thermometer has reached 100°, so that the steam may expel the air, which is a good non-conductor of heat. After the valve has been closed the temperature rises rapidly (in ten minutes) to 120°, at which point volumes of liquid up to 50 *cc.* are sterilised in ten minutes longer. Larger volumes receive a somewhat longer time.

At the close of the experiment the steam is let off very slowly, in small portions within five minutes, so as to avoid frothing and spirting of the contents of the sterilised vessels. The autoclave is especially useful for boiling agar.

§ 54. Many spores of bacilli have such an enormous resistance to heat that liquids containing them cannot be certainly sterilised at once except with high pressure steam. Thus the spores of the hay bacillus die only on heating for three hours to 100°, at 105° they perish in fifteen minutes, at 107° in ten minutes, and at 110° in five minutes. Globig came upon a red potato bacillus, the spores of which were not killed until they had been heated for six hours to 100°, or for half an hour to 110°. If we have not an autoclave at our disposal we heat the liquids to be sterilised to 100° for half an hour to one hour daily for several successive days, thus continually destroying the bacilli which have been developed in the meantime from the spores (fractionated sterilisation).

The higher resistance of the spores may serve to decide the question whether micro-organisms observed were present as bacilli or as spores. Whatever is still living, after being heated to 75° to 80° for a quarter of an hour, is in the state of the spore.

§ 55. In order to sterilise the hands with certainty, Fürbringer recommended the following successive operations:—

1. Careful mechanical trimming of the nails.
2. Brushing the hands for one minute with a brush, soap and hot water, especially the spaces underneath the nails.
3. Plunging the hands for one minute in alcohol not weaker than 80 per cent.
4. Washing and kneading the hands for one minute in solution of sublimate at one part per thousand, or carbolic at 5 per cent.

5. Wiping the hands on a towel which has been recently washed, or drying the hands by successive rinsing in alcohol and ether.

As an antiseptic in experiments on animals we use solution of sublimate at one part per thousand. If we hurt the hand in a dissection, bleeding should be assisted by pressure, or, if needful, by cutting, and the wound should be washed with sublimate at one per thousand. Solution of sublimate, used for surgical purposes, is mixed with 0·5 per cent. NaCl, and kept well closed and in a dark place to render it more permanent, and to prevent the precipitation of the sublimate by albumenoid substances.

In order to destroy old cultures in test-glasses, &c., it is best to cover them with concentrated hydrochloric acid. When the cultures have been steeped for twenty-four hours in this liquid it is poured off and hot water is poured upon them, or preferably the contents of the glasses are boiled in the steam-pot. The glasses are then emptied and thoroughly cleansed with water and a glass-brush.

§ 56. **The Nutrient Substratum.**—The most important nutrient media are the following:—

Broth (Koch's original method). 500 *grm.* beef are finely minced, covered with 1 litre of water, allowed to stand over night in an ice-closet or cellar, the turbid liquid is poured off, the meat is first loosely pressed by hand, and then pressed out more energetically either with a straining cloth, or, preferably, with a meat-press. The liquid obtained is made up to 1 litre, 10 *grm.* of pure, dry commercial peptone, and 5 *grm.* sodium chloride are added, and the mass is now boiled in a 2-litre flask in the steam-pot. After the lapse of thirty minutes the slight alkalisation[1] of the acid, dirty-coloured liquid is undertaken, using violet litmus as an indicator, boiling then for an additional hour so as to effect the com-

[1] N. K. Schultz recommends for the slight alkalisation normal soda-lye. The necessary quantity is ascertained by titrating a small specimen with decinormal-lye; phenolphthaleine is used as indicator. Alkali is added until a very faint rose coloration appears.

plete separation of the coagulable albumenoids. A small portion is then filtered off; if it is still slightly alkaline and remains clear after being boiled for some minutes in a test-glass, the entire quantity of broth may be filtered. If the specimen becomes slightly turbid on boiling, or if the reaction has again become slightly acid, the neutralised broth must again be boiled up in the steam-pot with the addition of a small quantity of soda, and the liquid is then filtered. The filtration can be very rapidly effected on a folded filter. The filtrate is made up to 1 litre, poured into a sterilised flask, and once more boiled therein in the steam-pot in order to destroy any germs which may have fallen into it from the air; or it is at once distributed in portions of 5 to 10 *cc.* in sterilised test-tubes and boiled up once more in them, an operation which is best repeated the following day.

At present, in most laboratories, the broth is boiled as follows: The minced meat is set, with the water, at once on the fire, boiled for some hours (about two), allowed to cool in order to separate the fat, rendered slightly alkaline, and filtered, hierauf sterilisiert wie oben beschrieben.

Sometimes the peptone is omitted, or there is added, along with the peptone, a 1 per cent. solution of glucose, &c. One litre broth has also been prepared from 20 to 30 *grm.* of Kochs' peptone, or from 5 *grm.* extract of meat, 5 *grm.* glucose, and 10 *grm.* dry peptone. In any case, the liquid must be neutralised, or rendered faintly alkaline.

Solution of extract of meat often contains spores which are very difficult to destroy. It must therefore be boiled up three times in the steam-pot, each time for one hour, at intervals of twelve hours. Sterilised milk is often used. New milk is sterilised by boiling for one hour in test-tubes closed with wadding and placed in the steam-pot. On the two next days the process is repeated for thirty minutes each time. As the cream rises the glasses, for many purposes, must often be shaken. In preparing these nutrient solutions, it is well always to have ready a number of test-tubes full of sterilised water, and a solution of NaCl at $\frac{1}{2}$ per

cent., as both are often needed.[1] If fresh skim milk is procurable, it is to be preferred, as we thus avoid the disturbing action of the cream.

§ 57. **Solid Nutrient Media. Potatoes** (Schröter).—Sound potatoes, not too mealy, are peeled, and cut in slices 1 *cm.* in thickness, everything being removed which does not appear healthy. The raw slices are placed separately in small sterilised glass boxes fitted with lids, and are then placed for one hour in the steam-pot, where they are at once boiled and sterilised (E. Esmarch). Others recommend to bore out, with a cork borer, cylinders of potato of the width of a test-glass, to bisect them diagonally, to place each half in a sterilised test-glass, and to boil them in steam. In order to prevent the lower end of each piece of potato from dipping into condensed water, the glasses are contracted a little above their lower end, or a plug of wadding is placed at the bottom of the glass.

Koch's earlier method, of sterilising the potatoes externally in their skins, and cutting them in half with a sterilised knife after boiling, has been abandoned, as the spores of the earth-bacilli (potato bacilli) readily escape destruction, and may subsequently endanger the cultures.

Stiff potato-pulp introduced into glass boxes affords a very fine, smooth substratum.

Potatoes generally react faintly acid (according to Täges, old ones are always acid, but new ones are always alkaline). If it is intended to neutralise them, the "Esmarch" slices are laid, for some time before boiling, in a 5 per cent. solution of sodium carbonate, or we use potato paste, to which any desired reaction is easily given.

§ 58. **Transparent Solid Mediums. Broth-peptone-**

[1] Other artificial nutrient solutions are rarely used. That of Pasteur contains to 100 parts water, 1 part ammonium tartrate, 1 part sugar candy, and the ash of 1 part yeast. Cohn's solution contains to 100 parts water, 0·5 potassium phosphate, 0·5 magnesium sulphate, 0·05 tribasic calcium phosphate, 1 part ammonium tartrate. According to another formula: 100 parts water, 8 parts glucose, 0·5 acid potassium phosphate, 0·5 acid ammonium phosphate.

gelatine (Koch).—At present the nutrient medium most generally used (for the reason see § 72).

We proceed as in the older methods of preparing broth (§ 56), but we add to the juice of the meat, before boiling, 0·5 per cent. of salt and 1 per cent. of peptone, 6, 8, or 10 per cent. of the finest gelatine, which must be first cut into pieces of the size of the little finger, that they may be more conveniently slipped into the flask. It is now heated in the water-bath, shaking occasionally, until all the gelatine is melted. It is rendered slightly alkaline with sodium carbonate or hydroxide (sensitive red litmus paper must be turned distinctly blue), and the flask is set for forty-five minutes or one hour in the steam-pot. If the reaction is still slightly alkaline or neutral, it may be filtered, which is effected through a folded filter moistened with water. As the gelatine readily congeals, it is well to use a hot-water funnel, *i.e.*, an apparatus in which the glass or copper funnel containing the filter is enclosed in a metal funnel filled with hot water. A small flame keeps this water hot. In default of such apparatus, it is advisable to use a glass funnel of medium size, pouring in little but very hot gelatine, and keeping the funnel covered with a glass plate. Cautious and momentary application of a Bunsen flame to the funnel much promotes filtration. The process may also be expedited by keeping the funnel covered externally with a thick woollen cloth.

When the gelatine is in a proper condition it filters readily, is clear as crystal, slightly alkaline, and congeals readily. In other cases—often, probably, if the boiling has been insufficient—it filters very badly, and constantly congeals again. If it has been boiled too long uninterruptedly, it loses the power of gelatinising, and is then useless. If the filtrate is not quite clear, the defect may be removed, if the liquid is too alkaline, by adding a small drop of acetic acid, and shaking up (resolution of basic phosphates): the liquid becomes clear as soon as the reaction is faintly alkaline.[1]

[1] The importance of the reaction of the nutrient medium is still not sufficiently kept in view. A. Reinsch (*Central. f. Bakteriologie*, x. 415) shows to what a great extent the enumeration of fungi in waters is affected by slight differences in the alkalinity of the nutrient media.

In other cases turbidity exists though the reaction is right, and repeated filtration is not of much use. In such cases we may add the whites of two eggs, shake well up, and boil for half an hour in the steam-pot, when the coagulating albumen carries down the turbidity.

The gelatine, filtered into a sterilised flask, is either closed up with wadding, or it is at once distributed, in portions of 5 to 10 *cc.* each, in sterilised test-glasses, and, after congealing, it is boiled up again on the two following days, each time for half an hour. A part of the glasses are then allowed to coagulate in a sloping position, so as to give gelatines with a larger surface. To others there may be added a few drops of aqueous solutions of methylene blue, litmus, magenta, &c. ("colour-tubes"), in order to study the behaviour of the bacteria with these colouring-matters. An addition of 1 to 2 per cent. of glucose, and, more recently, of 3 to 10 per cent. glycerine, is frequently resorted to. The colour of the meat-juice peptone gelatine is of a darker yellow the impurer the peptone, and of a paler yellow the purer it is. The proportion of gelatine in summer is often increased to 10 or even 15 per cent., to raise the point of coagulation. In winter many experimentalists work with 6 to 8 per cent. I find it more practical to work always with 10 per cent. The shorter the time for which the gelatine has been boiled, the less readily it melts in the heat of summer.

Wort gelatine is prepared by mixing unhopped wort, obtained from a brewery, with 10 per cent. of gelatine, boiled without neutralising, and filtered. Upon this slightly acid culture-medium yeasts and filamentous fungi flourish especially well.

§ 59. **Broth-peptone-agar.**—We prepare broth as in § 56; add 10 *grm.* prepared agar, torn up as finely as possible,[1] and

[1] Agar, formerly called agar-agar, is a preparation of marine algae from Eastern Asia: Gigartina and Gracilaria (Sphærococcus) consisting chiefly of a mucoid carbohydrate (gelose of the French). It is used chiefly when pressed into the form of loose quadrangular stems. The *Fucus crispus*, often recommended as a culture-medium in place of agar, has, in spite of several advantages pointed out by authors, not come into use.—*Centralblatt f. Bakteriologie*, viii. 281.

heat in the water-bath until the liquid is very hot. The dried flask is placed upon a wire gauze loosely covered with asbestos, and boiled at first over a small flame, and then over a larger one until the agar is dissolved. (Agar dissolves very slowly, and even in the swelled state it begins to melt only about 90°.) When the solution is complete it is made slightly alkaline, for which much less soda solution is necessary than for gelatine, which is in itself acid. Then to render the product capable of filtration ebullition is kept up over a small flame for three hours, replacing the water as it evaporates. Care must be taken lest the frothy liquid should boil over. The operation may be regarded as complete when yellowish white, dense flocks are readily deposited. The filtration can be effected only with the hot-water funnel and a peculiar thin filter-paper, and it is often a trial of patience. Agar does not always, like gelatine, become clear as crystal, but a slight opalescence is not at all injurious. For many purposes it is sufficient to filter through a compact woollen cloth, or a plug of wadding. If we can filter under increased pressure it is a great saving of time. It is best to place the filtering flask with the filled filter in the steam-pot or autoclave. Unna (*Centralblatt f. Bakteriologie*, ix. 749) recommends an especial apparatus, "a steam filter," for agar. The filtered agar is further treated like gelatine, but it may be boiled as long and as often as it is desired without losing its power of gelatinising.

If agar is to be used congealed in a slanting position, the tubes must be left for some time to lie undisturbed until the water has evaporated to such an extent that the agar remains adhering to the glass.[1]

Agar is unpleasant to work with, as after every liquefaction water always oozes out on congealing, which interferes with

[1] Recently proposals have been repeatedly made to improve the liquefaction and filtration of agar by a short soaking in acid (acetic or hydrochloric acid), followed by rinsing with water. N. K. Schultz (*Centralblatt f. Bakteriologie*, x. 58) states, however, that boiling agar in acidified liquids with subsequent neutralisation yields a product which gelatinises much worse than when the boiling is effected in broth which has been previously neutralised or which is slightly alkaline.

its adhesion to glass. An addition of 1 to 2 per cent. of gelatine, or the same quantity of gum arabic, remedies this defect.

Glycerine agar, *i.e.*, agar as above described, with the addition of 7 per cent. of glycerine, has been recommended by Nocard and Roux (*Annales de l'Inst. Pasteur*, i. 1887, No. 1) as an excellent nutrient medium for the bacilli of tubercle, and has rapidly come into use.

The chief difference between agar and gelatine nutrient media as commonly prepared is:—

1. Agar only liquifies at about 90°, and congeals at about 75°, it is therefore solid at the temperature of blood. No species of bacteria develops a ferment capable of liquefying agar.

2. Nutrient gelatine melts at about 23° to 25°, and at this temperature loses therefore the advantages of a solid medium. A number of kinds of bacteria liquefy the gelatine by their fermentation products. Pure cultures of this kind upon gelatine often acquire a very characteristic appearance on this account; plate cultures also deliquesce readily from the same cause.

§ 60. Along with the above a short mention must be made of a culture-medium which is more rarely used, *blood serum*. It is indispensable for cultivating certain pathogenic bacteria in a virulent form: the bacilli of tubercle and gonococci. The heart's blood of slaughtered cattle (especially wethers) is caught in sterilised cylinders about 20 *cm.* high, and 8 to 10 *cm.* broad, with well-fitting ground stoppers. For this purpose the breast is first well moistened around the wound, and only the second portion of the issuing blood is caught, as the first part is always accompanied with dirt. The cylinder is then stoppered and placed as quickly as possible in the ice-closet. After standing at *perfect* rest for twenty-four to thirty-six hours we obtain clear, yellow serum, and a solid coagulum.

With a sterilised pipette, loosely closed above with a plug of wadding, we lift off the clear serum and fill with it sterilised test-glasses in which we allow it to coagulate at 65° in

an inclined position, as shown in Fig. 28. Higher temperatures must be avoided, since above 68° the congealed mass is turbid, whilst at 65° it is clear and of an amber yellow.

The chest in which the coagulation is effected has double sides of sheet-iron, the interval being filled with warm water, and the whole closed with a glass cover. It must be very cautiously heated with a small flame, constantly observing a thermometer which passes into the air space within.

If the work has been carefully effected the contents of the majority of the tubes will be sterilised. To ascertain this point they are warmed three times, each for twenty-four

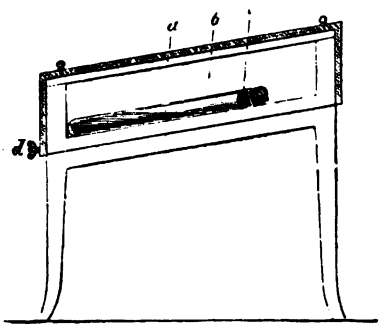

FIG. 28.—Apparatus for sterilising the serum of blood.

hours, from 30° to 35°; those which do not become turbid and develop fungi are certainly sterile and fit for use.

Formerly blood serum, before coagulating, was submitted, according to Koch's instructions, to a fractionated sterilisation; it was heated for six days in the apparatus to 58°, each time for one to two hours, when it still remained liquid.

Latterly, we find that with careful work and rejection of all the failures, 90 per cent. of the tubes in good experiments are sterile.

The higher the temperature of coagulation, and the greater the proportion of alkali and of saline matter, the more rapidly the serum coagulates; with increasing alkalinity the transparence of the coagulum increases, but its firmness decreases; with increasing temperature and higher proportion of saline matter the solidity and the opacity of the coagulum increase (Zoth, *Vienna Academie*, May 14, 1891).

Kirchner (*Zeit. f. Hygiene*, viii. 469) has recently made known a method for obtaining blood serum, which surpasses in convenience all former processes, and which is warmly recommended by Heim. The blood is received as above, though not in vessels which have been

especially sterilised ; the plasma after coagulation is cautiously detached from the sides of the vessel by means of a glass rod, whereby the yield of serum is increased. The latter is taken off from day to day as more collects for some days. The serum is introduced with sterilised pipettes into sterile medicine bottles holding about 100 *cc.* ; 1 *cc.* of chloroform is then added to each, and the bottle is closed with a boiled caoutchouc stopper, coated with paraffine. It is then allowed to stand some weeks, or preferably for two months (it keeps good for years), in order to be certain that the chloroform has destroyed all the micro-organisms in the serum, even those most tenacious of life. It is then filled into test-glasses, and left for some days in the incubation stove to allow the chloroform to escape. The serum can be coagulated as above, and used either in the solid or the liquid state.

Löffler adds to three parts of serum one part sterilised neutralised broth, with 1 per cent. peptone, 1 per cent. glucose, 0·5 per cent. sodium chloride. The coagulating power is thereby not affected, but the nutritive value for many bacteria is increased. An addition of 6 to 8 per cent. of glycerine is very useful (according to Nocard and Roux), it raises the coagulation point to 75° to 78°, so that more energetic sterilisation becomes possible (see below).

Serum gelatine, according to Koch and Hueppe, is liquid serum sterilised or received so as to be sterile, heated to 37°, and mixed with equal parts of 20 per cent. nutrient gelatine (or 2 per cent. nutrient agar) at the same temperature, and heated for some days daily to 52° each time for one hour. This mixture combines the advantages of gelatine and serum. It has the nutrient value of the latter, and, if cautiously heated, it can be liquefied and again solidified.

According to Behring, blood serum, diluted with five parts of water, can be sterilised by boiling without being rendered turbid.

According to Hueppe, raw eggs cleaned and externally disinfected with sublimate, then well washed with sterilised water and vigorously shaken up, form a good nutrient medium, which soon become free from oxygen. They may be infected through a tiny window in the shell, which, after the infection, is closed up with sterilised paper and collodion. Sterile white, or yolk of egg, received in a sterile glass is suitable for many species.

§ 61. A very large number of bacteria flourish and increase at a very wide range of temperature, from 10° to 12° up to 40°; others, *e.g.*, *Bacterium phosphorescens* (Fischer), grow even at 0°, and are greatly interfered with by 30°. Others, especially those occurring in the soil, begin to grow at 50°, and are not killed before 70°, even when free from spores.

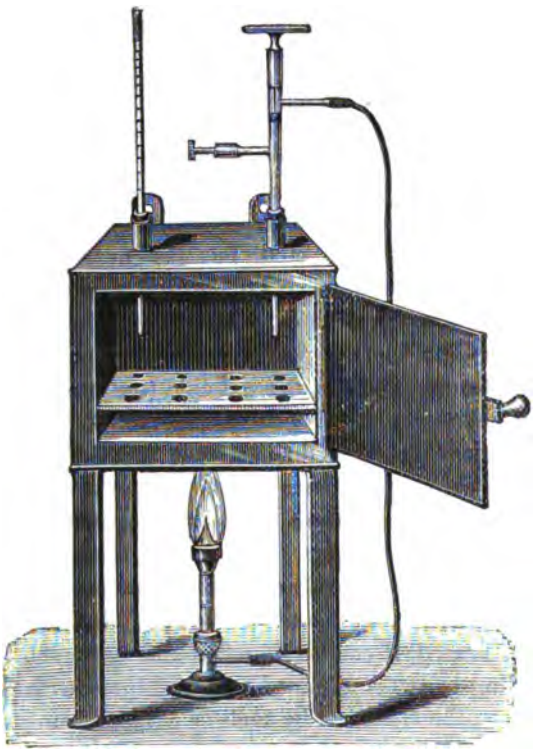

FIG. 29.—Simplest Chest for Heating or Drying.

The vegetation of most of the kinds hitherto cultivated has its optimum condition at about 25°, whilst numerous others, especially the pathogenic kinds, flourish best at 37°. In order to obtain a rapid formation of spores, this higher temperature should generally be selected.

Apparatus for producing these temperatures may be most simply constructed of large double-sided chests of sheet-iron

coated with lead. The spaces between the sides are filled with water, and the chests are covered outwardly with felt, or with wood and slag wool, or preferably with asbestos. Such a chest may be about 1 *m.* in height, 80 *cm.* in breadth, and 40 in depth.

If the water contained amounts to many litres, it suffices to keep a small gas flame burning under it by day and night, to maintain, in spite of occasional fluctuations of the pressure of gas, a constant temperature of 30° to 33°, or 25° to 28°. If a more exact regulation is required (to ½ or 1°), especially in cases of small thermostats (a primitive model of which is shown in Fig. 29), it may be effected either by a gas-pressure regulator, which keeps the pressure of gas approximately constant, enormous as are its fluctuations in most places, or we may make use of thermo-regulators; it is best to employ both these kinds of apparatus simultaneously.

§ 62. Gas-pressure regulators are chiefly intended to keep the supply of gas constant, whilst the pressure in the main varies. They are so arranged that the increasing pressure

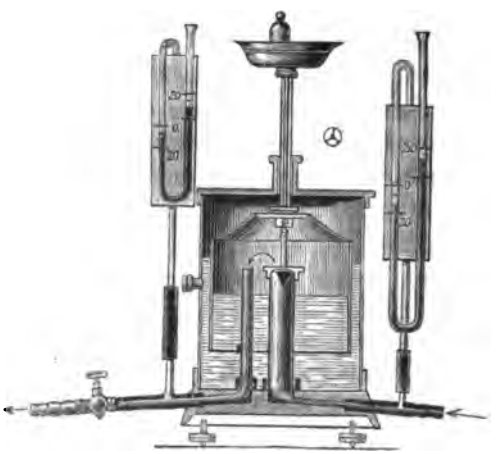

Fig. 30.

of the gas reduces the section of the supply pipe by means of an elastic membrane, or a conical valve actuated by a spring, so that, on a strong pressure, as much gas passes

through the narrow section as through a wider section at a lower pressure. In the pressure-regulator of Moitessier (Fig. 30) there is inserted in the gas current a bell, suspended in water or glycerine, which is loaded with weights, according to the mean pressure of the gas. From the middle of the bell there hangs a conical valve in the supply-pipe. If the pressure of gas increases in the pipes, the bell is raised, and with it the valve, so that the escape of the gas is diminished by the contraction of the aperture. Thus the height of the flame remains essentially unchanged. The apparatus must fit very accurately if it is to be of any service.

Thermo-regulators, which are in much more general use, aim at keeping the temperature of a given space constant, the temperature itself effecting an expansion of mercury, and thus reducing the temperature of the gas supply-pipe. A simple system is, *e.g.*, the following (Fig. 31, *b*): Take a wide tube (*Q*); expanded below, in the shape of an egg, there is sealed a narrower pipe (*E*). In the expansion there is placed a little mercury, and the apparatus is then placed in water which possesses the exact temperature for which the regulator is to act, *e.g.*, 70°.

The expanding mercury rises for a certain length in the inner tube (*E*), and the oblique end of the gas supply-pipe (*Z*), which passes through the stopper, is pushed so far towards the mercury that its point exactly enters. The apparatus is then set with its lower half in the space of air which is to be regulated, the gas supply-pipe is connected with the general main (not shown in Fig. 29), the exit tube with the burner (Fig. 29), and after the gas has been lighted, the temperature of the room, &c., is observed by means of a delicate thermometer.

The following action now ensues: By the rise of temperature the mercury expands, and closes more and more the opening of the gas supply-tube; the temperature rises then more and more slowly. The supply-tube must dip in so deeply that at a mean pressure of gas, and at the desired temperature, gas enough is delivered to keep the temperature

constant. As soon as the pressure of the gas rises appreciably, the flame becomes larger, the mercury rises a little; now the supply of gas decreases and the mercury sinks, the flame becomes larger again. The result of this play is that the temperature of the drying-closet rises a little, though not nearly so much as in the absence of a regulator.

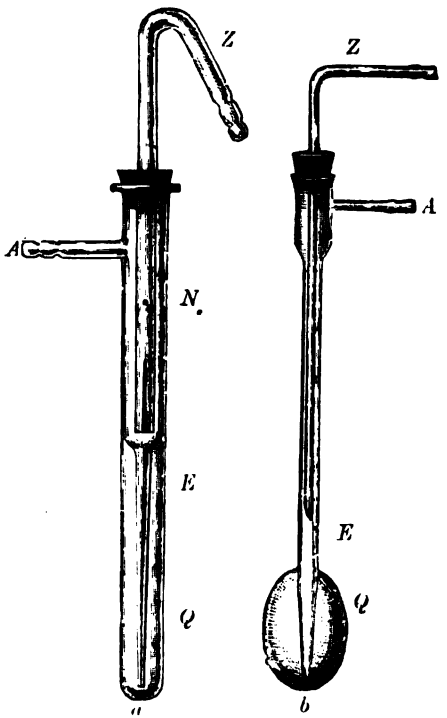

FIG. 31.—Thermo-Regulator.

If the gas suddenly rises much it may entirely close the aperture of the gas supply-pipe, so that only the safety-aperture *N* (Fig. 31, *a*) remains in action; mercury and temperature quickly fall, the ordinary aperture is set free, and the play repeats itself.

If the pressure of the gas does not fluctuate too greatly, and if the apparatus is carefully adjusted, it will keep the temperature constant to within 2° to 5°. Fig. 31, *a*, shows

a similar apparatus, though here the supply-tube *Z* has a slit instead of a point. The pressure of gas must of course not be so strong, and the safety-aperture must be so wide that even when the supply-tube is closed a flame still exists which raises the temperature. All regulators containing mercury must be inclosed in steel cases, otherwise a broken regulator may spoil an entire drying-closet by amalgamation.

The regulators of Soxhlet, now widely used, are also practical. In these, absolute alcohol serves as the regulating

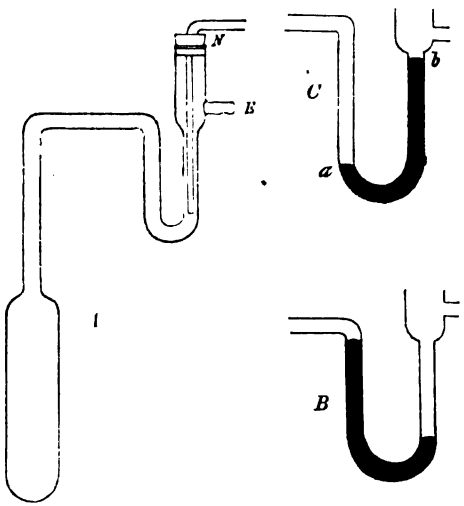

FIG. 32.—Soxhlet's Thermo-Regulator.

fluid, and mercury merely for closing. These are especially useful for low temperatures. The instrument is filled and set in action as follows:—

The expansion of the U-tube is closed at *N* with a stopper. The tube *E*, which is blown on laterally, is connected by a flexible tube and a glass pipe with the air-tube of the water-pump. We exhaust, close the connecting-pipe with the fingers, draw it away from the glass connecting-tube, and plunge it into a glass filled with absolute alcohol. After repeating this procedure, and suitably inclining, the apparatus, including the U-tube, is filled with absolute alcohol. The alcohol is now heated to ebullition by placing the regulator in hot water, so as to expel any absorbed air: the air-bubbles which have collected at the

upper elbow are removed by an inclined position. The regulator is now placed in a water-bath, the temperature of which is equal to the maximum temperature desired, representing, *e.g.*, 40°. After the immersed part of the regulator has assumed the temperature of the water-bath, so much mercury is poured into the upper opening that it may scarcely reach to the enlargement of the U-tube (Fig. 32, *B*). By slightly inclining the regulator, we allow a little alcohol to pass over from *a* to *b*, so that the mercury at *a* rises by about ½ *cm.*, and sinks as much at *b*. The alcohol which has passed over is removed with filter-paper. The regulator, when thus filled, is now to be used for the temperature aimed at on adjustment, and for 10° to 12° lower. Here, therefore, 40° to 30°, or 28°, the lowest temperature, is that at which the mercury takes the position indicated in Fig. 32, *C*. At higher or lower temperatures than those for which the instrument was adjusted it gets out of order, as alcohol passes over the mercury, or air makes its way into the space for alcohol. In order to adjust the regulator exactly to a given temperature, we proceed as follows: After the water-bath has been kept for a sufficient time at the required temperature, and the submerged part of the regulator has acquired the temperature of the water-bath, the gas supply-tube, which works with moderate friction in the perforation of the cork (not a caoutchouc plug), is approximated to the cup of mercury so far that the gas flame is of a medium size between the largest and the smallest. If, after some time, the temperature is by some tenths of a degree too low or too high, the gas-tube is displaced a little until the desired temperature is reached. The pressure of gas must be such that, if the supply-tube is not displaced, the flame is too large, but too small if the safety-aperture alone is in action.

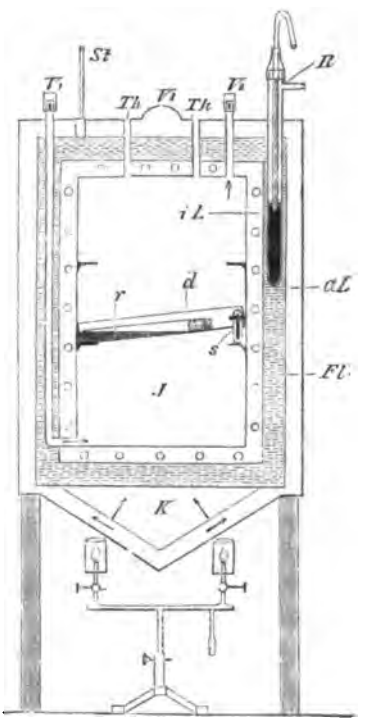

FIG. 33.—Hueppe's Thermostat.

§ 63. If this or any other more complicated regulator (which will not be here described) is adapted to the new thermostats, which are very suitably constructed, built of copper, fitted with conical or dome-shaped bottom, traversed

by ventilating channels, and are certainly very expensive, we may obtain an almost absolutely constant temperature, not differing by more than $\frac{1}{10}$th of a degree in several days.

An instance of such apparatus is shown in the accompanying thermostat of Hueppe's. The shaded space (Fig. 33) is filled with water, and entirely surrounded with an outer air-jacket, V_3, capable of being ventilated. The spacious incubation-room is supplied with ventilating pipes, V_1, V_2, and is protected by the internal screen, $i\,L$, against the direct temperature of the water.

In the d'Arsonval thermostats, much in favour in France, the expansion of the water-jacket of the incubation-room acts upon a caoutchouc membrane which regulates the influx of gas. A small, narrow ascending tube allows the volume of water to be kept very exactly equal, and thus keeps the temperature constant to $\frac{1}{10}$th of a degree.

Such absolutely constant thermostats are, however, necessary only for enfeebling bacteria, for delicate researches on the formation of spores, on modifications in the energy of fermentation, &c. An ordinary vegetating-case with a thermo-regulator, or, in case of need, even without it, suffices for the less refined purposes of practical hygiene.

An ice-closet remains necessary in every laboratory.

3. The Methods of Culture, and their Application for the Solution of Definite Questions.

§ 64. By means of the nutrient media described, the following questions may be solved:—

1. Counting the number of microbes present per unit-volume.
2. Isolation of the several kinds, and their pure cultivation.
3. Examination of their macroscopic and microscopic properties.
4. Examination of their biological peculiarities.
5. Examination of their metabolic products.
6. Examination of their pathogenic action.

§ 65. If it is required to ascertain the number of germs in a unit of volume, microscopic examinations are useless. It is easily effected if we succeed (Koch) in mixing the substance in question with the nutrient medium, so that all the schizomycetes are imbedded in the medium in a conspicuous manner, and distinct from each other. As each organism increases to a colony, objects, often of characteristic properties, are obtained capable of being seen and counted with the naked eye, or under a low magnifying power.

Manipulation.—We sterilise in the drying-chamber an accurate pipette, containing 1 or 2 *cc.*, and graduated in $\frac{1}{10}$ *cc.*, after it has been successively rinsed with sublimate, water (under the tap), alcohol, and ether, and finally dried by forcing air through with the hand-blast. In order to keep a pipette sterile we thrust small plugs of cotton wool into each end before heating. For use, the plug at the point of the pipette is removed; that at the upper end remains, so that when liquid flows out of the pipette, the air that enters is free from germs.

The contents of a gelatine glass are now melted at the lowest possible temperature (about 30°), or in the flame, and cooled in cold water down to about 30°: 1 *cc.* of the liquid to be examined, *e.g.*, branch-water, is sucked into the pipette, allowed to drop quickly into the gelatine by momentarily easing the plug of cotton wool, and mixed with the gelatine by gentle shaking. If we have no standard for estimating the number of microbes in the fluid, we infect three tubes with 0·1, 0·5, and 1·0 *cc.* (water, wine, beer, &c.): if a large number of microbes is suspected (sewage, sour milk, water in which soils have been suspended), a strong dilution of the original liquid is needful. This is best effected by means of a sterilised 0·5 per cent. solution of NaCl (100 parts to 10,000 parts), so that, on taking 0·1, 0·5, or 1·0 *cc.*, we may expect a number of microbes capable of accurate enumeration.

In order to bring the organisms separately to their full development, the following methods are in use, all known as *plate methods*, from Koch's original method, in which glass plates were employed, as described at the conclusion of this section.

1. For the experimentalist who works without special facilities, the most convenient is the capsule plate method, indicated almost simultaneously by a number of investigators, but generally ascribed to Petri.

The contents of the test-glass are poured completely, and as rapidly as possible, into a sterilised horizontal flat glass box, of about 7 to 8 *cc.* in diameter, the lid of which is lifted for a moment on one side, and is then immediately closed. The microbes then form separate colonies.

If great exactness is required, the test-glass is again closed with a cotton wool stopper, to ascertain if any colonies form in the remaining drops of gelatine.

The covers must fit well, and as a precaution the charged boxes are kept piled upon each other under large glass bells, lest aerial microbes may insinuate themselves between.

2. The roller plates (E. Esmarch) require no auxiliaries beyond the guarantee of a temperature in the room not exceeding 25°. After the liquid gelatine has been infected as above, a caoutchouc cap is placed over the plug of wool, and the test-glass, inclined almost in a horizontal position, is turned on its longitudinal axis under a stream of cold water, or laid in a dish of ice-water until its sides are coated with a stiff, transparent layer of gelatine. If a little gelatine has come upon the lower surface of the wadding, which it is desirable to avoid, we take off the caoutchouc cap, and thrust through the plug a stout, ignited platinum wire, in order not to interfere with the access of air.

The colonies of microbia may be easily counted after a few days, but those which liquefy the gelatine often interfere greatly, and still more does a high summer temperature.

Prausnitz (*Centralblatt für Bakteriologie*, ix. 129) has described a simple apparatus for completing ten roller plates at once. There are fixed upon an axle two round discs of sheet metal, at the distance of 14 *cm.* from each other, in which there are cut ten round holes, near the circumference, exactly of the width of the test-glasses to be used. We fix in the holes ten infected test-glasses, lay the axle over a

vessel of cold water, and cause the entire apparatus to revolve slowly by means of a handle fixed on the axle.

3. The original classical plate method of Koch may be mentioned mainly as having opened up an epoch. From it have been gradually evolved the convenient modifications here described. In its original form it has been generally abandoned as relatively complicated, requiring auxiliary appliances and the use of ice, and presenting few recommendations. Koch poured the liquefied gelatine upon sterile glass plates, accurately levelled and kept arranged in stages on glass bars under moist bells. The advantages of the capsule plates are especially great when many plates have to be poured at once (see § 66).

4. An apparatus has been recently described by several authors which is said to combine the advantages of the capsule plates and the roller plates. It is a bottle plate resembling a flat travelling flask. To my knowledge it has hitherto been little used.

§ 66. The enumeration of the colonies of bacteria on a plate is effected after forty-eight hours if they are very numerous, or if among them there are many which liquefy gelatine; but, if possible, not until the lapse of eight days at temperatures not below 15°, as the microbia which grow slowly do not earlier reach a visible condition.

The enumeration is effected by ascertaining the average number of microbia on a square centimetre and multiplying the number of the calculated surface (if the diameter is 8 *cm.* the superficies is $4 . 4 . 3\frac{1}{7} = 50\frac{2}{7}$ *scm.*). Koch's plate without a border is the most convenient for enumeration. It is laid underneath the counting apparatus of Wolffhügel upon a black plate of glass, the colonies upon the divided square centimetres are counted with the lens, and the mean of these numbers is taken. With capsule plates we proceed in a similar manner, but only such are suited for accurate computation whose bottom is perfectly level and permits the gelatine to be spread in an absolutely uniform layer. If the bottom is vaulted the stratum at the margin is much thicker,

and therefore richer in colonies. More than 100 to 200 colonies should not be present on a square centimetre if accurate enumeration is desired. If the number of microbia is very small we count all the colonies on the plate. Quite isolated microbia may always possibly be atmospheric impurities; the material to be examined is therefore diluted so that the number of colonies on the entire plate may be fewer than from twenty to fifty.

The roller plates, when they are close, require an especial enumerating apparatus, which cannot be described here. Thinly sown roller plates may be counted through and through with the aid of lines drawn with ink. They are

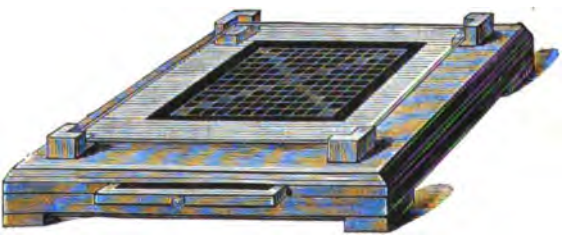

FIG. 34.—Counting Apparatus of Woffhügel.

especially to be recommended for the examination of substrata poor in microbia.

We must not forget that all enumerations on gelatine plates presuppose that *all the microbia* are able to develop themselves in gelatine; and, secondly, that each colony proceeds from a single individual. Both suppositions are inaccurate. We know already many schizomycetes which do not grow in gelatine, *e.g.*, the bacilli of tubercle, and we know many not at all, or not closely, because they do not grow in gelatine.[1] Further, single individuals are often united in a single growth.

There are few other substances for the preparation of plates. Agar must first be cooled down to 40° before it is infected. Agar plates, which are best arranged in boxes, have the advantage that the plate can be placed in an incu-

[1] Thus, *e.g.*, the tunnel of the Mangfall Water Works at Munich is lined for long distances with vast gelatinous masses of schizomycetes, among which an enormously thick rod-like growth is the most striking. It will not grow in gelatine, and consequently cannot be detected by the best methods known.

bation-chest, which is often very convenient. Further, agar is not liquefied by any of the known schizomycetes. Agar, to which a few drops of gum arabic have been added, to make it adhere the better to glass, is useful also for roller plates. Glycerine agar plates are selected for rearing schizomycetes, which are especially difficult to cultivate. Latterly, blood serum, mixed with gelatine or agar, has been used for plates. Liquid (not congealed) serum, and liquefied 2 per cent. agar cooled down to 40°, are not mixed until after infection.

§ 67. The second even more important application of plates is in *obtaining pure cultures*. If we examine a well-managed plate which, *e.g.*, has been arranged with 1 *cc.* of water, we see at once that the single small colonies present differences which are manifest even to the naked eye. There rest upon the solid gelatine little milk-white buttons, beside them flat, leafy, elegant colonies, and others which resemble yellow drops of wax. Others, again, have liquefied their neighbourhood, and sit in the midst of a small depression-like or flat funnel, filled sometimes with a colourless, sometimes with a greenish yellow liquid, &c. On a plate there may often be found ten or more easily distinguishable species. As each colony proceeded from a single individual (or possibly from a small group of conjoined growths), it is sufficient (Koch) to touch them separately with a sterilised platinum wire, to plunge the wire into a glass of congealed, sterilised nutrient gelatine, to readjust the plug of wadding, in order to have a pure culture (plunge culture). If the plate is thinly sown, and if the colonies are tolerably large, the simple method of inoculation just described proves sufficient. If numerous small colonies lie close together, the incculation has to be performed under the microscope.[1]

[1] In order not to obtain too thick plates, if our purpose is not enumeration but the examination for certain kinds, *e.g.*, of blood for the bacilli of splenic fever, we fit up several plates in the following manner. We inoculate, firstly, a little blood in the first glass ("original"), shake it round, put three wire loops-full of it into a second glass ("first dilution"); three loops-full of this in a third glass ("second dilution"). One of these three tubes is certain to yield a useful plate. It is often not necessary to use the original when we know that it is too rich in microbia, *e.g.*, in the examination of fæces.

If we bring a plate under the microscope with a magnifying power of about sixty diameters, the colonies often present characteristic features which escape the naked eye. They display sharply defined, toothed, fringed, ramified outlines, a granular or a smooth surface, &c., and we may thus often succeed in distinguishing two species which appear macroscopically identical.

If we find an especial colony from which we wish to form a pure culture, we bend the end of a platinum wire of moderate thickness into a very short hook, like a fishing-hook (1 to 2 *mm.* in length), and endeavour with the aid of the microscope to touch with the point of the hook the colony in question, and no other. For this purpose the "fishing" right hand is firmly supported upon a rest rather lower than the stage of the microscope, the needle is applied to the object lens with its point turned towards the operator, the point is slowly lowered, turning the hook away, and plunged for a moment into the colony. It is then drawn back quickly and carefully, and thrust at once into a gelatine tube. The whole operation requires a steady hand and moderate practice, but it is then not very difficult. The process is facilitated by means of a small auxiliary apparatus devised by Prausnitz (*Centralblatt für Bakteriologie*, ix. 129).

If it is required to isolate a certain microbe out of a mixture, it is plain that we must first ascertain as accurately as possible its appearance upon the plate, for which purpose preliminary plates must be prepared from pure cultures obtained elsewhere. If the growth upon the plate is not very characteristic, or if it is unknown to the investigator, tubes must be infected from a great number of colonies which may possibly consist of the microbe in question. The pure cultures thus obtained must be compared in their growth with each other, and, if possible, with pure cultures which have been accurately determined.

§ 68. In order to isolate micro-organisms which do not grow upon a solid medium, the liquid in question must be so largely diluted that a small quantity, *e.g.*, 1 *cc.*, may only

contain one germ. We ascertain for this purpose by the microscopic enumeration of a dyed dry preparation the number of microbia which may be present, in *e.g.*, $\frac{1}{10}$ *cc.* of the original solution (or of the solution after dilution to a known extent).[1] If 100 micro-organisms are found in $\frac{1}{10}$ *cc.*, 1 *cc.* must contain a 1000; hence 1 *cc.* must be diluted with 999 *cc.* sterilised liquid, so that each centimetre may contain one germ. Of this dilute liquid we pour 1 *cc.* (containing one spore) into each of very many test-glasses or flasks of a suitable nutrient solution; the majority of the cultures obtained will be pure, and some of them will contain the

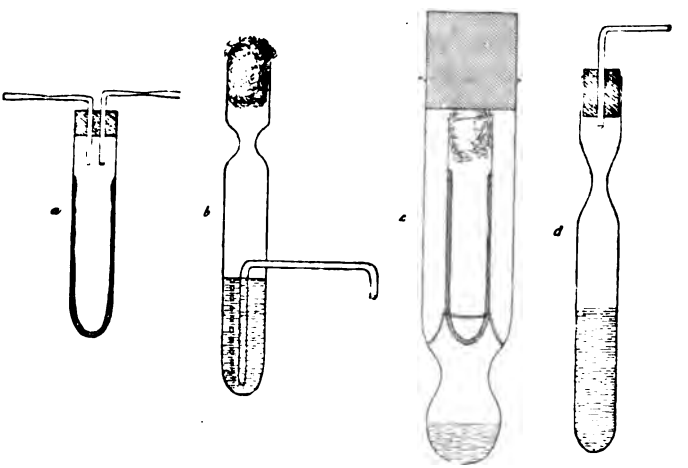

FIG. 35.—Methods of Anaërobic Culture.

micro-organism sought for. As a nutrient solution the sterilised liquid is frequently used from which the microbe is to be isolated, *e.g.*, sewage, urine, milk, &c.

Into the use of the methods of diluting microbes for computation (Miquel), as much approved in France, I shall

[1] Much more accurate determinations of the number of microbia are obtained by means of "microscopic counting chambers," *e.g.*, those of Thoma-Zeiss. The liquid containing the microbia fills a low space $\frac{1}{10}$ *mm.* in height; a glass cover with a fine graduation allows the number of microbia to be ascertained very accurately by counting some fields of $\frac{1}{100}$ square *mm.* superficies and 0·1 in thickness. The micro-organisms are counted in the undyed condition.

not here enter. Those interested are referred to Hüppe's description (p. 268, &c.). Its great complication, and its demands on time and room, cause this method to appear almost useless for practical purposes, even if really larger numbers of microbia are obtained than by plate-culture upon solid nutrient media. In Germany the process is scarcely employed at present.

§ 69. A number of schizomycetes flourish, as it was discovered by Pasteur, unlike the great majority, only in the complete absence of oxygen (anaërobic microbia). For obtaining pure cultures of these organisms the following three methods, founded upon the principle of Esmarch's roller-plate, or of the shaker culture, have been for a long time in exclusive use. A nutrient gelatine is used containing 2 per cent. of glucose. When tubes are to be used in these experiments they must be drawn out to capillaries at the intended points.

1. According to Hüppe and Fränkel. Liquefied gelatine is infected in a test-glass closed with a caoutchouc stopper having two perforations, through which are passed two angular bent glass tubes. Through the longer tube there is passed for fifteen minutes a current of pure hydrogen, so that the bubbles traverse the gelatine, which is kept in a water-bath at 25° to 30°. In conclusion, the glass tubes are melted off whilst gas is still passing, and the gelatine is distributed as a roller-plate on the sides of the vessel. Very similar was the much used method of Liborius-Flügge, passing the hydrogen as shown in Fig. 35, *b*, and then melting off the gas delivery-pipe and the test-glass. It is less safe but more convenient to prepare the roller-plate, to set it in ice-water, to expel the air by means of hydrogen, and to melt off the tubes (Fig. 35, *a*).

2. According to M. Gruber (Fig. 35, *d*). The infected gelatine is placed in a glass with a drawn-out neck in a water-bath of from 30° to 35° (agar must be kept at from 41° to 42°), and the air is evacuated with the water-air pump after inserting a well-fitting caoutchouc stopper traversed by

a glass tube. The removal of the air is assisted by watery vapours which escape out of the gelatine as it boils at the reduced pressure. The condensation of water must be prevented by passing a Bunsen flame along the narrow neck, and after about fifteen minutes it is melted off. The unrolling is effected at first without refrigeration until the vacuum is saturated with water.

3. According to Hans Buchner (Fig. 35, *c*). 1 *grm.* pyrogallic acid and 10 *cc.* of a 3 per cent. potassa-lye are placed at the bottom of a wide glass, a small wire support is introduced, and upon it is placed the roller-plate with the plug of wadding slightly loosened. The external glass is then closed with a well-fitting caoutchouc stopper, and in twenty-four hours all the oxygen has been consumed by the alkaline pyrogallic acid, so that anaërobic species can now begin their growth.

Of these methods, that of Buchner seems to me the most convenient. It may, indeed, sometimes fail us in case of anaërobic species, which are killed by the access of even small quantities of oxygen, but in very many cases less sensitive spores will also be present. We also find, perhaps, in the course of the culture, many anaërobes which are not very sensitive to the access of oxygen.

Quite recently we have universally come over to the plan of casting ordinary box-plates, and letting them grow in air free from oxygen. For this purpose nutrient agar with 2 per cent. of glucose and a small percentage of gelatine is used.

Since the arrangement of boxes under the air-pump, and the introduction of hydrogen into boxes with ground glass covers fitted with connecting-tubes, has been found impracticable, Blücher (*Zeit. für Hygiene*, viii.) recommends a simpler method, which has been improved by Botkin (*Zeit. für Hygiene*, ix.). They place in a glass trough over each other a vessel with alkaline pyrogallic acid, and three to four charged box-plates, whilst a glass bell is inverted over the boxes. Into the trough is passed paraffine oil (liquid vaseline), and two flexible tubes, strengthened with copper wire,

are conveyed into the space within the bell. One of these tubes serves for the influx and the other for the efflux of a powerful current of hydrogen for fifteen minutes. The flexible tubes are then removed, and any remaining traces of oxygen are absorbed by pyrogallol.

In my Institution my assistant, Dr. Arens, has obtained the finest results by filling an ordinary Bunsen exsiccator (Fig. 5, *a*) in its lower part with coarse gravel, adding a tablespoonful of pyrogallic acid and 30 *cc.* of 3 per cent. soda-lye, and placing then upon the stratum of gravel in the upper part of the apparatus one to three flat box-plates. The lid of the exsiccator, which is ground to fit, was afterwards thoroughly pasted over outside with paraffine, and the apparatus was placed in the incubation-closet. All the proved anaërobia grew splendidly, and aërobia were absent.

There occur, moreover, all manner of transitions between species which are compulsorily aërobic, and such as are occasionally anaërobic, thence to the occasionally aërobic, and finally to the necessarily anaërobic species.

§ 70. The plunge-cultures for aërobia to be laid out in gelatine or agar are best prepared by plunging once only to the depth of 3 to 5 *cc.* with a platinum needle of medium strength. If gelatine glasses have been filled for some time, the gelatine, which has been partially dried, easily cracks on inserting the needle. Such glasses are therefore melted once more before use, and allowed to cool. The different kinds of plunge-cultures present highly various aspects. We know colonies of schizomycetes of all colours; some, requiring oxygen, grow on the surface, others (averse to oxygen) grow either not at all without especial expedients, or flourish only in the lower part of the mass. The majority, however, grow both on the surface and in the aperture made by the needle. Many liquefy the gelatine owing to the formation of ferments, whilst others leave it solid. Many evolve gas-bubbles, odoriferous bodies, &c.

Upon agar the growth is less characteristic, since it is not liquefied by any microbe. All the schizomycetes which

liquefy gelatine grow upon agar in thickish masses, but various pigments are formed upon it in abundance. Potato-cultures often produce very characteristic growths, possessing great diagnostic value. Plunge-cultures from agar are especially suitable for the preservation of pure microbia for subsequent use. If we inoculate fresh agar every one to two months, we need not fear that our cultures will perish.

Anaërobic micro-organisms can in general also be raised without difficulty in 2 per cent. sugar-agar, preferably with the addition of glycerine. The test-glass is filled to the depth of 6 or 8 *cc.*, and the plunge or insertion is made with a long thin platinum wire bent at the end to a loop (Kitasato). In this manner it is easy to introduce the infectious matter into the deepest parts of the medium to be infected. The cultures are placed in the incubation niche, and grow to within about $\frac{1}{2}$ *cm.* of the surface, but ultimately even this extent may be almost entirely overgrown under the influence of the gases generated.

For peculiarly sensititive anaërobic species, we may, according to Löffler-Fuchs, after infection invert the test-glass, pass through it a powerful current of hydrogen, and quickly insert a caoutchouc stopper, which has been previously boiled, and which may be coated externally with paraffine.

For preserving the cultures it is best to use a closet or wooden chest with a folding lid, in which one or more large reagent-stands with cultures may be set. In this manner the cultures are protected from dust, and from too great heating of the sun's rays, which exert a destructive action.[1]

The infection of a fresh test-glass with pure culture is effected as follows: The surface of the plugs of wadding in the glass *A*, from which the infection is to be taken, and *B*, the one to be infected, are carbonised in order to destroy any micro-organisms which may have fallen in. Both plugs are

[1] For preservation it has been recommended to develop cultures upon potatoes, to cut quickly out of them, with a sterilised knife, small pieces, which are placed in sterilised test-glasses: a carefully preserved culture on agar remains good equally well.

eased by twisting; *A* is seized between the thumb and the forefinger of the left hand with its mouth downwards, the plug is eased for a moment, a freshly ignited but cooled platinum wire is brought in contact with the culture, the plug is again secured, and the glass is set aside. *B* is then quickly opened with its mouth downwards, the needle is inserted into the gelatine, the plug is secured, and the needle is again ignited.

Tubes with liquids can merely be held slanting, but not inverted. Even so a contamination of the air is very unlikely if we take care not to stir up dust during the process of infection. The transfers are preferably effected when the operator is alone in the room, and walking about should be avoided.

§ 71. If certain proof is required that a microbe which we have isolated is identical with one already described, the task is easy if it possesses very characteristic properties, *e.g.*, *Bacillus prodigiosus*. In such a case the following investigations are sufficient, which it is best to carry on simultaneously with cultures of a correctly determined microbe obtained from trustworthy hands, and treated in an absolutely identical manner:—

1. Appearance of the gelatine plunge-culture, examination of colour, odour, liquefaction of gelatine, manner and rapidity of growth. If it is found that the organism does not grow upon gelatine at the temperature of the room, it is tried upon glycerine-agar, blood-serum, &c., using the incubation-closet to induce development.
2. Appearance of the potato-culture.
3. Appearance of the gelatine plate colonies, macroscopically and microscopically at sixty diameters.
4. Appearance when highly magnified.
   a. Dyed.
   b. Undyed in suspended drops. Examination as to spontaneous movements, formation of spores, &c.
5. Experiments in infection (see § 73).

In difficult cases, when it has been shown that there are

several species analogous to the one supposed to have been found, *e.g.*, typhus bacilli, or if a precise diagnosis of a new species is required, further investigations must be added to those mentioned above. Here I must limit myself to indications.

6. *Examination upon Coloured Gelatine.*—Coal-tar colours (magenta, methylene blue, phloxine) are added in watery solution in small quantities to the fully prepared gelatine, so that it is brightly coloured. Many colonies of bacteria grow up of an intense colour, withdrawing the pigment from the gelatine, whilst others absorb no colouring matter. Methylene blue and litmus are decolorised by reduction by all the microbia which liquefy gelatine. On shaking up the colour is transiently restored.

7. Cultivation in nutrient gelatines, with the addition of alkalies, acids, glycerine, phenol, &c. The nearly allied species display often very characteristic differences with injurious additions.

Cultivation in liquids. Chemical examination of the products of metabolism, *e.g.* :—

a. Culture in broth or in Cohn's solution, &c. Examination for the formation of nitric acid, nitrous acid, ammonia ; for the formation of free acid.

b. Culture in milk. Examination for the formation of lactic acid, butyric acid, &c. ; observation whether the milk coagulates in a clotty, flocculent, or in a gelatinous state ; whether the coagulation is effected by the formation of an acid, or in a neutral condition, by means of a ferment like rennet ; whether the coagulations are subsequently peptonised, and whether pigments are formed. A tryptic ferment has been detected in case of the cholera vibrio. Concerning the detection of bacterial ferments see *Centralblatt für Bakteriologie*, viii. 203; Claudio Fermi, *Archiv für Hygiene*, x. and xiv. Lauder Brunton, and Macfadyean.

c. Culture in solutions of glucose (or in broth containing glucose). Examination for formation of acids, gases, &c. Careful investigations may here bring to light the most interesting facts. Thus we know schizomycetes which produce inactive fermentation lactic acid. Nencki has informed us of others which yield dextro-rotatory lactic acids (carno-lactic acid and para-lactic acid), and recently Schardinger has obtained a lævo-rotatory lactic acid—hitherto quite unknown—the optical counterpart of dextro-lactic acid, as the product of special bacteria in a solution of glucose. See Nencki, *Centralblatt für Bakteriologie*, ix. 304.

d. Culture in glucose and milk sugar broth coloured with litmus. Many microbes redden the colouring-matter of litmus, others turn it blue, and others decolorise, *i.e.*, reduce it so that the colour, red or blue, returns only on shaking with air. The

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formation of acid or alkali depends in part on the composition of the nutrient medium. The presence of sugar especially promotes the formation of acid. See Smith, *Centralblatt für Bakteriologie*, viii. 389.

e. Cultivation on starch paste. The formation of sugar proves a diastatic function. In other cases cultivation in beer-wort, in urine, in white of egg, &c., may give interesting results.

f. Examination whether indol, phenol, hydrogen sulphide, &c., are formed.

1. *Detection of Phenol.*—The broth culture is distilled after one-fifth of its volume of strong hydrochloric acid has been added. A flocculent precipitate on the addition of bromine water to the distillate proves the presence of phenol or cresol.
2. For the demonstration of indol we make use either of cultures upon meat juice, peptone, broth, or preferably upon the fresh sterile uncooked flesh of a rabbit. The latter cultures are lixiviated with water and boiled with a little acetic acid in order to remove the albumenoids. A few cubic centimetres of sulphuric acid (diluted with three parts of water) are added, and the whole is covered with a solution of sodium nitrite at 0·01 per cent. At the surface of contact of the two liquids there appears a ring of a rose colour or cherry colour. (See Kitasato, *Zeit. für Hygiene*, vii.; Petri, *Arbeiten des Kaiserlichen Gesundheits Amtes*, vi.; Kuhn, *Archiv für Hygiene*, xiii.)
3. Sulphuretted hydrogen is detected by suspending in the space of air over the culture a slip of paper saturated with lead acetate, or sodium nitro-prusside rendered alkaline. The former reagent is turned brown and the latter violet.

g. Examination for the formation of poisonous conversion-products. The culture boiled, or preferably filtered through earthenware cells, is used for experiments in poisoning.

8. Examination of the optimum, maximum, and minimum temperatures for growth, the resistance to desiccation, &c.

9. Examination of the property of forming spores. Smear-cultures upon agar are placed in an incubation niche at 37°, and an examination, morphological and physiological, is undertaken of any spore-like formations which appear. They have to be examined under the microscope both when recent and when dyed. Threads of silk saturated in the sporiferous matter are dried in the sulphuric acid exsiccator. At the same time a portion is heated for half an hour in a

test-glass, which stands in a water-bath at 70° to 80°, after which, if spores are formed, a culture made up with them shows its growth.

10. Latterly some other nutrient media have been recommended, but they have not been sufficiently used to require here a description in full. Kühne (*Z. f. B.*, xxviii. 1890, p. 172), Winogradsky (*C. f. B.*, x. 209) and Sleskin (*C. f. B.*, x. 209) propose gelatinous silica; Beyerinck (*C. f. B.*, ix., 781) chalk gelatine; Kaufmann (*C. f. B.*, x. 65). infusion of jequirit.

As examples of such investigations may be mentioned: Hans Buchner's "Contributions to the Knowledge of the Naples Cholera Bacillus, &c." (*Archiv für Hygiene*, iii. 361); and Weisser, *Ueber die Emmerichschen sogenannten Neapler Cholerabakterien* (*Zeitschrift für Hygiene*, i. 314).

§ 72. **Appendix to Chapter III.**—The following are the uses of the chief nutrient media:—

a. Liquids:

1. For the study of the products of transformation, the susceptibility to fermentation.
2. For studies on microscopic morphology.
3. Exceptionally for obtaining pure culture of microbia which will not grow on a solid nutrient medium.
4. For producing pure cultures with an equal number of microbia per cubic centimetre or per drop.
5. For mass cultures, as the results from a test-glass of broth are different from those of a small glass of gelatine.
6. For differential diagnosis.

b. Solid nutrient media:

a. Gelatine and agar:

1. For plate-cultures, *i.e.*, for the demonstration, the accurate separation, and the enumeration of individuals and species.
2. For obtaining characteristic macroscopic cul-

tures, which serve for differential diagnosis (agar for the formation of spores).

3. For permanent cultures, or collections of living bacteria.

β. Blood-serum and glycerine-agar:

For rearing especially pathogenic species, which on other media grow with difficulty or not at all. Plate-cultures are possible only with glycerine-agar and mixtures of agar and serum.

γ. Potatoes:

1. For obtaining cultures of great permanence macroscopically characteristic, and for differential diagnosis.
2. Occasionally for the formation of spores.

Gelatinous transparent nutrient media meet with the most extended application, on the following grounds:—

They are simultaneously applicable as liquids and as solid media. As liquids they allow of the separation, and as solids of the fixation of the isolated germs, and of their separate growth to form colonies.

On account of their transparency they permit of both a macroscopic and a microscopic consideration of the cultures installed: they allow far-reaching differential diagnosis of the species, and a prompt recognition of impurities.

Along with the gelatine and the agar nutrient media, liquids, solid blood serum, and potatoes retain their place as important and often indispensable adjuncts.

4. Experiments with Schizomycetes on Animals.

§ 73. From various intimations given in the course of our exposition, it appears that pure cultures of pathogenic species which are difficult to suit with a substratum generally grow slowly in comparison with the saprophytic species. Thus the bacilli of splenic fever, or of tubercle, are often difficult to obtain by means of lifeless nutrient media among mixtures of microbia, especially when they are present in small

numbers along with numerous saprophytes. In such cases the inoculation of susceptible animals often exceedingly facilitates their recognition. Scattered bacilli of splenic fever in a nutrient medium are most readily demonstrated by the fatal splenic fever which occurs in guinea-pigs or mice after being inoculated with a specimen; with the bacilli of tubercle the case is similar. As a matter of course the demonstration of a microbe which occasions an infectious chronic disease presupposes that there is not another microbe present which kills rapidly, such as the bacillus of the septicæmia of rabbits, of malignant œdema, of tetanus, &c.

§ 74. For testing the pathogenic effect of a schizomycete we have the following methods:—

1. *Subcutaneous Inoculation.*—A place on the skin of the animal, (rabbit, guinea-pig, &c.) is carefully shaved, disinfected with sublimate at $\frac{1}{1000}$, washed with sterilised water, and a flat cut is made with scissors through the skin and into the wound, which scarcely bleeds. A little of a culture is introduced by means of a platinum wire ending in a loop. A mouse is inoculated by grasping it firmly at the point of the tail with the crucible tongs, drawing it up to the edge of a glass, holding the tail fast with the left hand, and with the right covering the glass with a board, so that only the tightly-drawn tail and the point of the rump project. On the posterior side of the base of the tail the hair is cut off, the skin moistened with solution of sublimate, washed and dried, and a small flat pocket is made in the skin, by means of a lancet, into which the infectious matter is introduced.

In many cases infection is effected with spores (*e.g.*, splenic fever). Splenic fever is allowed to form abundant spores upon agar in the incubation-closet at 35° to 37°, which requires from twenty-four to forty-eight hours; sterilised silk threads of 1 *cm.* in length are then rolled in the infectious matter in the drying-closet. The moist threads, which adhere to each other, are spread singly out with forceps upon a sterilised plate of glass, and they are then allowed to

dry whilst covered with a glass bell. When well dried such threads keep for years, and always form a good material for inoculation.

2. A modification of subcutaneous inoculation is effected in the anterior chamber of the eye. With a small, sharp knife and a lancet we open the anterior chamber close to the sclerotic margin, separating the cornea from above, as in iridectomy. The point of the knife penetrates first obliquely backwards: as soon as it has arrived in the anterior chamber it is carried obliquely forward, and drawn back in the same manner. This method is especially practised for the diagnosis of tubercle on the eye of rabbits. A small quantity (a loop) of the suspected sputum, tissue, dust, &c., is inserted through the section. After some weeks the characteristic

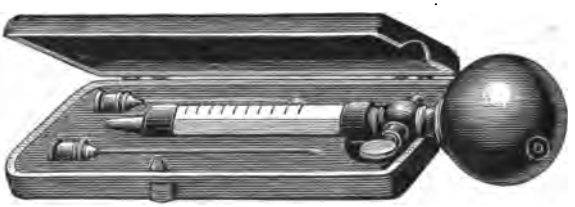

FIG. 36.—Koch's Injection Syringe.

tuberculosis of the iris is developed, and subsequently general tuberculosis is set up. Inoculation in the peritoneum of guinea-pigs, especially in case of the bacilli of tubercle, is more convenient.

3. *Cutaneous Inoculation* is the name given to the procedure when it is effected as in vaccinating children; only the cutis but not the subcutaneous connective tissue is severed, and the microbia are introduced—a method little in use.

§ 75. 4. *Subcutaneous Injection.*—For this process we use most commonly the syringe represented in Fig. 36, as it is easily sterilised, and works without piston, and with a caoutchouc ball and a cock. The sterilisation of the glass and metal parts is effected in the drying-closet from 140° to

150°. The ball is not sterilised. If it is contaminated it is laid in sublimate, filled by momentary compression, rinsed out with hot sterilised water, and dried in the room. The ball is screwed on to the syringe, the point is fixed in its place, the ball is compressed whilst the cock is open, the point is thrust into the infectious liquid, the ball is allowed slowly to expand, and the cock is closed when the syringe is full. The ball, which is still somewhat compressed, is screwed off, and again screwed on in its expanded state. The point is inserted in the direction of the folds, raising up a longitudinal fold; examine if the point has entered and if it is movable, open the cock, and compress the ball slowly, until the desired quantity of fluid has been forced in. The syringe is then unscrewed, and, if needful, filled again. Three to six cubic centimetres of liquid can be easily injected at the same place. If it is intended to inject more (experiments in poisoning), the place of insertion is varied (the region of the flanks on both sides, before and behind, the back, or the belly).

In the same manner we inject, in special cases, the muscles, the pleura, the lungs, the trachea, the peritoneal cavity, the joints, beneath the *dura mater cerebri*.

Injections are rarely made into the duodenum (to avoid contact with the gastric juice). The belly is shaved, sterilised, the duodenum is drawn out with sterilised forceps through a cut in the *linea alba*, and injected through the coat of the bowel, as above. Not a drop must be allowed to enter the peritoneal cavity. The duodenum is then returned to its place, the wound stitched up with sublimated silk, and a light bandage of sublimated wadding is applied, with gauze bandages.

An excellent syringe, cheap, easily sterilised, and readily managed with one hand, is due to Stroschheim (*Centralblatt für Bakteriologie*, vii. 746), of which I have made frequent and satisfactory use. The simple advice of Tavel (*Centralblatt für Bakteriologie*, v. 550) is very useful:— Any unsterilised syringe whatever is connected by an unsterilised flexible tube with a sterilised glass tube drawn out to a point at one end. In the other end is placed a plug of wadding. If the piston is drawn up, the liquid enters

the sterilised point, which may be calibrated. The plug of wadding must remain dry.

§ 76. 5. *Intravenous Injection.*—This less usual method is performed on an animal which is carefully tied down. The *vena jugularis externa* is laid free for the length of 2 to 3 *cm.*, a ligature is applied as near the heart as possible, the peripheral part of the vein is raised up upon a string, cut open, and a glass tube is inserted. This tube is then connected with the injection-syringe by means of a short flexible tube filled with a sterilised solution of sodium chloride at 0·6 per cent., and the injection is performed. The glass tube is then untied, cut off, and the wound in the skin is stitched up. In rabbits it is simplest to pierce the sterilised skin obliquely with the point of a modified Pravaz syringe into a vein of the ear, and to inject carefully.

6. *Introduction into the Stomach.*—In rabbits and guinea-pigs an elastic human catheter is passed into the œsophagus through the block of a gag which is placed cross-wise in the mouth (or, without gag, through the lateral gap in the teeth whilst the mouth is kept closed). In rabbits this is not difficult if we keep as close as possible to the vertebral column, and take care not to get into the trachea. It is more difficult in the guinea-pig, where only very narrow catheters can be introduced. The catheter is purified by forcing through it a 1 per mille solution of sublimate, and subsequent syringing with water.

The injection is performed with a larger syringe, of the construction already mentioned. Material containing spores often leads readily to infection (splenic fever), whilst bacilli free from spores are harmless, as they perish in the acid gastric juice.

7. In many cases infection may be conveniently introduced by feeding with potato-cultures. Mice and rabbits feed most readily if they have been allowed to fast for twenty-four hours previously.

The possibility that microbia may effect an entrance into the system through small injuries on the snout, in the

mouth, &c., may be almost absolutely excluded as follows: In little cubes of potato or albumen, into which a section has been made parallel with one of the surfaces, we bend back the laminæ thus formed like a lid, scoop out the block, fill it with matter scraped from a culture (gelatine, agar, or potato), close the lid again, and place it at the back of the tongue, so that the block is swallowed without chewing. The protective action of the gastric juice Koch eliminates, in case of guinea-pigs, by neutralising the contents of the stomach with 5 *cc.* of a 5 per cent. solution of soda. The vibriones of cholera act from the stomach for some time after injection, after 1 *cc.* tincture of opium for 200 *grm.* of the weight of the guinea-pig has been introduced into the peritoneal cavity to annul the peristaltic motion.

8. Experiments in infection by breathing are rarely performed. See Hans Buchner in *Archiv für Hygiene*, viii. 145, where may be found an accurate description of experiments in the inhalation of spores and vegetative forms, with all the precautions, a close observation of which alone can render such observations of value (reproduced *in extenso* by Hueppe, p. 365, &c.). The organisms can be introduced, along with water, in the form of spray, or they may be dried up with dust. Especial caution is needed in working with species which are pathogenic for man. The simplest and most recent apparatus is described by Buchner (*Centralblatt für Bakteriologie*, vi.).

§ 77. In order to prevent accidents, infected animals must be preserved most cautiously, so that they may not escape, and their excreta must be accurately collected.

Mice are placed in glasses about 15 *cm.* high and 10 *cm.* in width (such as preserved fruits are sold in), which should be daily changed, and provided with a handful of wadding and a piece of a roll steeped in milk. The cover consists of a strong, close wire gauze, which is bent down elastically over the projecting margin of the glass.

Rabbits, guinea-pigs, and rats are best kept in cages, the bottom and sides of which consist entirely of quadrangular

iron frames covered with wire netting. The cage stands on four feet, on a flat tray of sheet-iron, painted in oil-colour, and filled with peat-mull. The excreta are thus deodorised: The peat-mull, which is changed daily, is burnt, and the sheet-iron tray is disinfected with solution of sublimate, one part per thousand. Dogs and cats are kept in the same manner. Before every new experiment the entire cage must be cleansed mechanically, and after it has been thoroughly moistened with sublimate solution it must be brushed out with the same solution, and then rinsed with water.

Concerning the state of the animals, observations must be made and recorded. Determinations of temperature are made in the rectum, taking care that the thermometer is introduced to an equal depth in each case. The high normal temperature of many animals must not be disregarded. As soon as an animal is dead, dissection must take place at once. If this is not practicable, the body is quickly laid upon ice in the ice-closet. The results of the autopsy of an animal which has been dead for some time, and has been kept in a warm place, can be used only with great caution.

§ 78. In deciding on the results of experiments in infection, the following considerations must be kept in view:—

If an inoculated animal betrays no susceptibility for the supposed vehicle of infection, this may be because the species under observation enjoys immunity against the microbe in question, *e.g.*, field-mice are proof against the septicæmia of house-mice; house-mice are proof against glanders, to which field-mice are very susceptible; dogs are almost proof against splenic fever, &c. On the other hand, there are animals of the same species more or less susceptible; a negative result on one animal proves, therefore, nothing except it is repeated with the same result on from two to four other specimens. But the failure of an attempted infection may have also other causes, *e.g.*, an erroneous manner of infection. Cholera bacilli in rabbits and guinea-pigs are efficient only in the stomach and the intestinal canal; bacilli of splenic fever, when free from spores, act only in the lungs or by a sub-

cutaneous or intravenous infection. Various methods should always be tried, unless—as it is often the case in hygienic investigation—we are concerned with one only method of infection. For instance, it is not sufficient to show that a fungus supposed to be injurious if eaten occasions diseases if injected into the blood. Everything depends here on its effects if we adhere to the *natural* way of infection. It needs no demonstration that inoculations must not be made by thrusting a glowing needle into the culture, and so scarcely obtaining a single reliable schizomycete. If repeated experiments on animals of different kinds, effected in different manners, have given no positive results, we are not yet entitled to pronounce the microbe to be non-pathogenic for man. Thus, *e.g.*, there have been obtained with leprosy only doubtful results, and with typhus, gonorrhœa, and syphilis hitherto never positive results, on the lower animals; there are, therefore, organisms pathogenic for man, for which none of the lower animals is known to be susceptible. On the other hand, man cannot take cattle-plague (rinderpest) or chicken-cholera.

There are, therefore, cases where we must pronounce a microbe to be an exciter of infection solely because it—

1. Is found in every case of the infectious disease in question which has been examined at the proper stage.

2. Because the distinctly characterised organism in question is found nowhere except in the disease concerned in man (or some lower animal), or in its surroundings.

§ 79. If we have obtained positive results in the series of experiments sketched out above, it still does not at once follow that we have before us an exciter of infection. We call a microbe infectious only if, when introduced into the body in minimal quantities, it can produce a characteristic infectious disease—local or general—by its multiplication in the body of the animal. A second animal must also be capable of infection from the first patient.

If we require large quantities for infection, and have always had failures with small doses, two portions of

100 to 200 *cc.* each of broth should be infected with the microbe, and the one portion, after it has been sterilised by boiling for half an hour in the steam-pot, and the other after it has been freed from all microbia by filtration through an earthen cell, are each used for injections. Very often the liquid sterilised in one of these manners acts as well as before, which proves that the result was due to the action of the transformation-products of the microbe, and must be regarded as poisoning, not as infection.[1]

Sterilisation by filtration is effected as follows: The

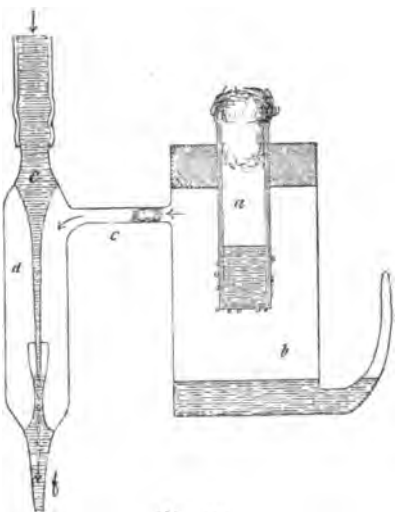

FIG. 37.

sample of broth in which the microbe has been developed is poured into the earthen cylinder, *a*. The latter is fixed firmly, by means of a caoutchouc stopper, in the glass cylinder, *b*, provided with two appendages (Fig. 37). The one appendage is melted on and drawn out, the other is open, and is provided with a plug of wadding, *c*. The air in

[1] It must be remembered that dissolved alkaloids, ferments, &c., are at first kept back by the clay-cell filter, and that portions of a full virulence can be obtained only after a large quantity of the liquid has passed through (Sirotinin-Flügge).

the glass cylinder is rarified by means of a water-air pump, *d*, *e*, *f*, fixed in at *c*, which causes the liquid to pass slowly through *a*. The filtrate is free from microbes if the apparatus had been previously sterilised by a dry heat, and the cylinder is free from minute chinks. The filtrate may be poured out by breaking off the point of *b*: a plate cultivation allows the absence of germs to be ascertained most simply. The expensive porcelain filters of Chamberland are scarcely more effective. Reichel has recently devised an especially suitable form of the clay-cell filter (*Verhandl. der Würzburg Phys.-Med. Gesellschaft*, 1891).

§ 79*a*. The isolation of the poisonous products of the transformation of matter in many bacteria is still in its initial stages. Until recently the view prevailed that the basic nitrogenous substances (monamines, diamines, triamines, ammonium bases, &c.) were to be examined to explain the poisonous action of bacteria. Brieger's beautiful researches ("On Ptomaines," Parts I., II., III. Berlin, 1885–1887) had made us acquainted with a series of such organic bases, which were known universally as putrefaction-alkaloids, or ptomaines, as the first such bodies had been isolated out of putrescent mixtures. The poisonous ptomaines were distinguished as *toxines*. A good conspectus of these bodies is to be found in Kobert's *Toxikologie*, 2nd edit., 1892. See also Jacquemont, *Les Ptomaines*, a monograph to which a prize was awarded, Brienne, 1890; a separate reprint from the *Journal de Medecine, de Chirurgie, et de Pharmacie*, 1890, No. 18.

The toxines isolated from the cultures of pathogenic bacteria according to Brieger's method[1] are often not capable of furnishing a satisfactory toxicological explanation of the disease. Indeed, in some especially important infectious

[1] The masses are extracted with weak hydrochloric acid, and the concentrated extracts are precipitated with sublimate. This precipitate is decomposed with H_2S, the filtrate is concentrated and extracted with alcohol. Purification is effected by means of the gold and platinum double salts, which serve for determining the crystalline form, for analysis, and for experiments upon animals. Some bases are not thrown down by sublimate.

diseases (splenic fever, diphtheria) specific basic matters, have been found either not at all or in vanishingly small quantities.

A new idea has been introduced into the study of the poisonous products of metamorphosis by Hankin (*British Medical Journal*, Oct. 12, 1889). He has isolated a poisonous albumose by precipitation with alcohol from cultures of splenic fever in meat-extract, with the addition of fibrine. The product obtained was applicable for protective vaccinations against splenic fever.

Hankin's pupil, Martin (Proceedings of the Royal Society of London, May 1890), obtained similar results, quoted in the *Centralblatt für Bakteriologie*, viii. 376. But along with the poisonous albuminoids he isolated also some bodies resembling the alkaloids. Such poisons (to be classed with the albuminoids) we meet with in plants, *e.g.*, the ricine of Kobert; the poisons of serpents may also perhaps be here included.

Very important researches have been made in this direction by L. Brieger and C. Fraenkel (*Berlin Klin. Wochenschrift*, 1890, Nos. 11 and 12). They obtained from broth-cultures of the bacilli of diphtheria, of tetanus, of typhus, from cultures of staphylococcus, and from watery infusions of the organs of animals suffering from splenic fever, exceedingly poisonous albuminoids, which they call *toxalbumines*.

The method was, filtration through a Chamberland filter, then either precipitation with ammonium sulphate, dialysing the filtered precipitate to remove excess of saline matter, dissolving in water, concentration in a vacuum at 40°, or, evaporation of the filtrate in Chamberland's filter down to one-third of its volume, and precipitation with alcohol and a little glacial acetic acid. By repeating this process there is obtained a white light powder, which in case of diphtheria, splenic fever, and tetanus is readily soluble in water, and approximates more to the albumines; but if from cholera, typhus, staphylo-coccus, it is sparingly soluble or insoluble in water, and approaches the globulines.

Petri (*Annalen des Kaiserl. Gesundheits' Amt*, vi.) and

Scholl have isolated from cultures of cholera, and Scholl also from those of *Proteus*, *toxopeptones* the poisonous properties of which are in part diminished by heating to higher temperatures, but in part little or not at all.

Finally, we learn from Buchner (*Centralblatt für Bakteriologie*, vii. 1; viii. 322; ix. 416) the important fact that the bacterial proteines obtained by Nencki from the bodies of bacteria (the microbial masses of agar- or potato-cultures are scraped and dissolved in hot potassa-lye at $\frac{1}{2}$ per cent. and precipitated with dilute acetic acid) are very powerful promoters of inflammation and suppuration. These proteines, which resist heat very well, exert in the organism a directly attractive action upon the leucocytes. Of course a sharp line of demarcation cannot always be drawn between the products of metamorphosis and the constituents of the contents of the cells.

More than these indications cannot at present be given within the limits of this work, as every week brings to light new researches based upon these very promising commencements. Into the use of these various substances for prophylactic and curative vaccinations (which have been already attempted with success), I must not allow myself to enter. The *Centralblatt für Bakteriologie* can be recommended as giving a better insight. Beginners in chemical research will scarcely effect anything of value in this region, as the method formerly recommended for the isolation of the products of bacterial metamorphosis (*i.e.*, shaking out with ether in acid or alkaline solutions, with benzine, chloroform, &c.) is now rarely applied. See Dragendorff, p. 108.

§ 80. The examination whether a schizomycete has multiplied or not in an inoculated animal is easy in extreme cases. If a proof has to be furnished a definite number of schizomycetes will be used for infection, *e.g.*, 1 *cc.* of a solution the contents of which have been enumerated.

In the dead animal, the blood, any pathological exudations, the liver, kidneys, &c., ground up very finely in broth, will furnish material for plate-cultures, the enumeration of which,

along with somewhat circumstantial weighings and computations, will enable us to ascertain the approximate number of microbia.

An approximate indication whether an increase has taken place in acute infectious diseases is given by the following experiment:—

Two animals are simultaneously infected with an ascertained number of microbia; plates are made up with the blood and the ground organs of the one half an hour after the infection, and with the blood, &c., of the other animal twenty-four hours afterwards, or at its death. If multiplication has taken place a decided increase of microbia will be observed in the plates from the second animal.

If the blood of living men has to be examined bacteriologically, a perforation may be made with a needle or a lancet in the skin, which has been previously carefully cleaned (§ 55). If more blood is required it is preferable, according to Scheurlen, to insert into a vein, in a direction opposite to the current of the blood, a fine glass tube drawn out at one end, and closed at the other with wadding. The investigation by means of plate-cultures yields much more trustworthy results than can be obtained by means of the microscope alone. The vein which has been perforated is then bound up with iodoform wadding. That all the instruments employed must have been sterilised by heat is a matter of course. (See Scheurlen, *Centralblatt für Bakteriologie*, viii. 257; and Smith, *op. cit.*, ix. 49.)

§ 81. A few words may be added on taking sections with bacteriological precautions.

The animal is tied down with the four extremities, the back downwards; the hair on the belly is thoroughly shaved away, the hairs are carefully removed, the shaven skin is thoroughly moistened with solution of sublimate (one part in a thousand), or, preferably, the entire animal is immersed in solution of sublimate and disinfected.

There are now arranged under a glass bell a number of forceps (at least four), knives (two or three), and scissors

(two or three), which have all been previously packed in a sheet-metal box in the drying-closet, and sterilised at 140°. In addition two sterilised vaccination needles. There have been previously set in readiness a number of gelatine or agar and serum glasses, and three or four small glasses of absolute alcohol; six to twelve covering-glasses, which have been carefully cleansed and taken through the flame, are laid in rows upon white paper and covered with a glass bell.

During a dissection an assistant is very useful who wipes the instruments which the operator lays in carbolic acid at 5 per cent., or in boiling water, sterilises them again in the flame, and takes notes, &c.

The epigastric coats are severed with a sterilised knife along the *linea alba* as far as the peritoneum; this is then cautiously laid open with a fresh knife; portions of the peritoneal secretion, if any, are put upon two covering-glasses and in two gelatine tubes, the intestine is briefly inspected, and a piece of the liver is quickly cut off. A deep cut is then made on the surface of the liver with a fresh sterilised knife, then a deep cut with a fresh knife at right angles to the former; small particles of the innermost substance of the liver are taken by means of a platinum loop from the surface thus laid bare, and we at once put it into gelatine tubes. The kidneys and the spleen are treated in the same manner. Pieces of the organs of the size of a hazelnut are then laid in absolute alcohol, and streak preparations on cover-glass are then made with the juice of the organs. The covering-glasses, which have been streaked, are then placed under the glass bell to dry, putting to each a label showing the organ with the juice of which it has been obtained. The cavity of the thorax is then opened from the diaphragm upwards with a fresh knife, and there are taken in succession specimens of the pericardial liquid, of the pulmonary secretion, and the blood of the heart for cultures and microscopical examination; further portions of the lungs and heart, and small pieces of the stomach, the bowel, the lymphatic glands, the skin, &c., are preserved in alcohol for section. Blood from the heart is obtained by opening the

pericardium antiseptically, cutting into a ventricle with a knife, and inserting platinum loops into the heart. Or instead we may introduce into the heart a sterile glass tube drawn out to a capillary point, and sealed and closed with a plug of wadding at the other end. The point is then broken off, whereby a larger quantity of blood is obtained, which may be emptied out by blowing into it. It is often convenient to inoculate a fresh animal directly with the blood or the organic juices. As soon as the dissection is completed the body of the subject is destroyed perfectly by combustion in a powerful fire, *e.g.*, in the furnace of a large steam boiler. If this is impracticable the body is wrapped in clothes well wetted with solution of sublimate, a hole is dug in an inclosed spot to the depth of ½ *m.*, a layer of quicklime is put in upon which the corpse is laid, and again covered with quicklime.

The gelatine-cultures laid out are at once converted into roller or capsule plates, the gelatine having been very cautiously liquefied in hot water at 35° in order that the microbia may not be injured by excessive heat.[1] Along with the original plates dilutions are of course installed (§ 67, note). Infected agar does not admit of any subsequent liquefaction.

If we have to search for pathogenic organisms in a corpse which is already putrescent (which is only done in case of need), the parts are laid for ten minutes in carbolic acid at 5 per cent., and then for five minutes in a solution of sublimate at 1 per cent. (containing 0·5 per cent. tartaric or hydrochloric acid). The surface is then rinsed with sterilised water, and cut open with sterilised knives, which are still hot, and central portions are torn out with forceps, thrusting the inoculation needle into them. The utmost caution is necessary in interpreting the results.

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[1] Many keep liquid gelatine and agar at hand during dissection by means of hot water, and cast the plates immediately after infection—a method which has its advantages.

5. Conspectus of the Most Important Schizomycetes.

§ 82. **Prefatory Remark.**—This conspectus comprises all kinds which are more accurately known, and which occasion important infectious diseases in man or in other animals; among the zymogenous, chromogenous, and saprogenous species I have mentioned, along with those of most practical importance, some of those which are used by preference for practice in university curricula. For determining species it is generally necessary to possess gelatine plate-cultures, plunge-cultures in gelatine and agar, potato-cultures, sometimes broth- and milk-cultures. Each experiment in determination is preceded by a careful macroscopic and microscopic study of the cultures. Very few kinds are perfectly characterised by single features, the majority must be determined by the sum of the individual characters. The various morphological and physiological properties of bacteria are modified by cultivation. We have bacteria of splenic fever which is no longer pathogenic, of splenic fever which has lost the power of producing spores, bacteria which no longer set up fermentation in milk-sugar, various chromogenous species which grow temporarily or permanently colourless; the rapidity of the liquefaction of gelatine varies enormously in cultures of the same kind. The mutability of single features must compel us to bestow a many-sided study upon each species if we wish to carry out determinations, or to draw from the properties observed conclusions as to their practical signification. Very many species named in literature, or characterised by letters, have been so imperfectly described that their recognition from the description is impracticable. A strict systematic description is as yet absolutely impossible; many species are only very loosely defined, and as their limits must be regarded as quite provisional, much will have to be explained in the next few years.

For the determination of the species which occur less frequently, the books enumerated in the bibliography, especially the work of Eisenberg, are indispensable; but a conscientious determination will often not admit of a certain

diagnosis, even if all existing authorities have been consulted, and the species found must be provisionally regarded as new. On the other hand, there are certainly numerous species which have been described by different investigators under different names, and which are identical, or are merely to be regarded as varieties. It is remarkable that we can easily, by artificial interference, deprive bacteria of their pathogenic, chromogenic, zymogenic or sporogenic functions, but that it is generally impracticable to restore these properties if their absence has lasted for a series of generations.

Abbreviations: G. = broth-peptone gelatine, containing about 8 to 10 per cent. of gelatine. A. = broth-peptone agar. P. = potato. W. = growth.

I. Conspectus of the Aerobic Species which may be Cultivated at 20° upon Meat-juice-peptone gelatine.

A. Cocci.

§ 83. Strictly globular or, at most, short oval cells in different arrangements; never any approach to the form of rods or of filaments. The formation of spores has been verified only in a few sarcinæ and micrococci. Spontaneous movements have been traced hitherto only in one flagellated micrococcus. (*M. agilis*, Ali Cohen.)

I. Cells united in cubic conglomeration (8, 16, 64, more rarely 4), like bales of merchandise. Short oval forms are more frequent than globes. *Sarcina.*

1. G. is rather slowly liquefied in the form of a funnel; yellowish brown upon G.; upon A. and P. orange; cells small; common in air. *S. aurantiaca*, Koch.
2. G. liquefied very slowly; sulphur yellow upon G., A., and P.; single cells very large; common in air. *S. lutea*, Flügge.
3. G. remains solid; cells very small (1 to 1½μ); upon G. a grey, slow, irregularly ragged surface-growth; scarcely any growth below; scanty and colourless upon P. and in B. In the respiratory tract. *S. pulmonum*, Virchow, Hauser.
4. G. remains solid; cells large; upon G. there occurs only the formation of 1, 2, and 4 cellular groups. In infusion of hay there then appear brownish packages, arranged cubically; yellowish white upon G.; chrome-yellow upon P. In the stomach, especially if dilated. *S. ventriculi*, Goodsir, Falkenhayn.

II. Cells combined by fours in a plane, united to families by a cement or sheathing matter incapable of being stained; in no stage cubic colonies.

Upon G. a juicy white surface-growth as a flat deposit; in a plunge-culture there appear minute lumps and granules of a moderate size; G. is never liquefied.

Upon A. little that is characteristic; upon P. a thick slime that can be drawn out in threads. Colourable by Gram's process.

Fatal to white mice in three to six days; guinea pigs are attacked with abscesses; rabbits, grey mice, and dogs enjoy immunity. According to Karmarsch it excites suppuration in man. *Micrococcus tetragonus*, Gaffky.

Lindner has recently discovered three species which grow upon all nutrient media in tetrads but not in cubic groups. They grow in beer, and they have been formed into the genus *Pediococcus;* especially *P. acidi lactici* (Lindner) forms lactic acid in abundance (*Centralblatt für Bakteriologie*, iv. 427). The genus *Pediococcus* seems to correspond with the older genus *Merismopedia.*

III. Cells connected like links of beads, but this type of growth becomes distinctly observable only in broth-culture. Upon G. and in organs it often occurs in single individuals in small heaps and in short chains. *Streptococcus.*[1]

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[1] The strangles in horses—an infectious catarrh with swelling and suppuration of the lymphatic glands of the neck—is certainly produced by a streptococcus, according to Schütz and other authors. This species, according to Schütz, grows on serum only (*Centralblatt für Bakteriologie*, v. 14). Poels asserts that it grows with difficulty upon G. and A. Hell also refers the pulmonary disease of horses to a streptococcus (*Centralblatt für Bakteriologie*, viii. 365), whilst Schütz and Fiedeler ascribe it to an organism resembling Friedländer's pneumobacillus.

Von Lingelsheim (*Zeitschrift f. Hygiene*, x. 1891), working at Koch's Institute, has compared nineteen different cultures of streptococcus, and arranges them in two groups:—

	In Broth (Microscopic.)	In Serum of Ox-blood.	Gelatine.	Potato.	Virulence.	In Broth (Macroscopic.)
Streptococcus longus.	Chains of as many as fifty links.	Long chain.	No liquefaction.	No growth.	Exists, but is readily lost.	Forms small clots.
Streptococcus brevis.	Short chains, rarely ten links.	Long chain.	Liquefied.	Grey-white films in incubation stove.	Entirely absent.	Uniform texture.

To *Streptococcus longus* belong: *S. erysipelatis* and *pyogenes*, concerning the distinctions between which he does not explain himself further.

Streptococcus brevis is probably related to the *S. coli gracilis* of Escherich. See *Annalen des Kaiserlichen Gesundheits Amt.*, vii.

1. On the G.-plate very tender colonies finely granulated, clear as water, forming round, drop-like groups. In the G. plunge-cultures it develops slowly; a tender, limited, white surface-growth, and a white, finely granular growth in the track of the needle; the cultures which lie more deeply become subsequently of a greenish brown. Upon A. a grey, superficial growth, thin at the margin; upon P. a scanty growth after the medium has been rendered alkaline with soda. In broth turbidity owing to luxuriant growth. It can be stained according to Gram's method.

   Here belong a series of closely connected species, which are probably, for the most part, merely different grades of virulence of one and the same organism.

   In the skin (especially in lymphatic fissures) in erysipelas in man. *Streptococcus erysipelatis*, Fehleisen.

   In pus in 50 per cent. of cases. *Streptococcus pyogenes*, Rosenbach.

   Organisms of this class have been observed by Flügge in a leukæmic spleen (*S. pyogenes malignus*); by Hartmann in puerperal infections in pelvic phlegmons; by Löffler, Ruskin, and others as an exciter of secondary infection in diphtheritis and scarlatina; by Nicolaier and Guarneri in soil. The more virulent forms kill mice, produce erysipelas or phlegmons on subcutaceous innoculation into the ears of rabbits, and even kill rabbits.

   See Bender, *Centralblatt für Bakteriologie*, iv., where may be found the entire literature of the question whether these allied species are distinguishable.

2. G. liquefies very quickly, grows rather luxuriantly; sparingly upon A. Always in putrid flesh, not pathogenic. *Streptococcus coli gracilis*, Escherich.

3. The chains of streptococci are surrounded by an enormously thick coating of gelatine (Dextran, a carbohydrate); numerous individuals coalesce to thick lumps of jelly. In the waste waters from sugar works, sometimes interfering with the manufacture, as it greedily consumes sugar. *Leuconostoc mesenterioides*, Cienc.

IV. Every pair of hemispherical cells are connected by a narrower or broader band of a substance which cannot be stained, resembling the shape of a German roll (*semmel*). Many authors give the name of Diplococcus to all globular or oval schizomycetes, or to those in shape like a short rod arranged in pairs, e.g., *Diplococcus pneumoniæ*, A. Fränkel.

   Here follow, along with *Diplococcus gonorrhœæ* (§ 86), some harmless saprophytes.

   G. slowly liquefied; culture a splendid rose colour; an aërial microbe very common in Würzburg. *Diplococcus roseus*, Bumm.

   Further, Bumm's *D. albicans amplus*, *D. albicans tardissimus*, and others.

V. Microscopically without any especially characteristic arrangement, predominant tendency to form irregular lumps and heaps. To this group belong all the cocci which cannot be arranged in the four preceding groups.[1]

1. G. is liquefied.

a. Liquefaction slow; the cultures have an outline with rounded indentations and sink gradually into the G. On agar thick deposits. Can be stained according to Gram's process.

Injection into the veins occasions purulent nephritis and inflammation of the joints; subcutaneous injection produces formation of pus in rabbits, osteomyelitis if a bone has been injured, and endocarditis if the valves of the heart have been interfered with. Found in every true pus, in phlegmons, boils, &c.; sometimes in sewage, air, &c. In milk it forms lactic and butyric acids. The virulence of the staphylococci fluctuates greatly. Transitions between the three following species have hitherto not been demonstrated :—

1. Upon A. gold colour to orange; paler on G.; formation of pigment distinct only after the third day; white in absence of oxygen. *Staphylococcus pyogenes aureus*, Rosenbach.[2]
2. Upon A. and G. white. *Staphylococcus pyogenes albus*, Rosenbach.
3. Upon A. and G. lemon-yellow. *Staphylococcus pyogenes citreus*, Passet.

Flügge has described a series of saprophytes which liquefy slowly, and which have in part a very characteristic growth upon gelatine, *e.g.*:—

Micrococcus radiatus, Flügge.

Micrococcus flavus desidens, Flügge.

b. Liquefaction rapid.

Here belong a number of aërial and aquatic microbia, hitherto of no practical importance, *e.g.*:—

Micrococcus coronatus, Flügge.

Micrococcus ureæ liquefaciens, Flügge; converts urea into ammonium carbonate.

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[1] A number of microbia of this group, which inhabit pus, have been arranged by authors, following the example of Ogston and Rosenbach in the sub-genus *Staphylococcus*, which in my opinion should either be abandoned or extended to numerous microbia, which I have included in V. Further, the staphylococci of pus, if strongly magnified, display in many cases the "semmel" form, and might therefore rank among the diplococci (Bumm, Heydenreich, *Centralblatt für Bakteriologie*, v. 59).

[2] The fundamental bacteriological researches on pyogenous species in man are: Rosenbach, *Mico-organismen bei den Wundinfektionskrankheiten*, Wiesbaden, 1884; Passet, *Untersuchungen über die Aetiologie der eitrigen Phlegmone*, Berlin, 1885.

2. G. not liquefied.

Here belong very numerous species common in air, water, milk, soils, &c., which can scarcely be said to have any practical importance, *e.g.*:—

Micrococcus candicans, Flügge, } form thick white deposits upon G. like drops of stearine.
Micrococcus ureæ, Leube and Graser, }

Micrococcus aquatilis, Flügge.

Micrococcus cinnabareus, Flügge.

Micrococcus pyogenes tenuis, Rosenbach. Rather larger than the staphylococci, without characteristic arrangement, white; very tender upon agar; found in abscesses; pyogenic (§ 86). Perhaps identical with *Diplococcus pneumoniæ*, Neumann and Hägler.

B. Bacilli.

§ 84. The longitudinal diameter is at least one and a half to twice the breadth, more frequently three to four times. Upon suitable culture-mediums many kinds are disposed to grow to filaments. Formation of spores has been certainly observed in many cases, whilst in others it is as certainly absent. In other species the observations instituted have not been sufficiently numerous.

I. The sole form of growth is a short rod one and a half to two breadths in length; two rods are often connected together, less commonly four. Formation of spores is wanting in most species. (Corresponds approximately to Cohn's genus *Bacterium*.)

1. G. is not liquefied.

A. Culture upon G. white, without a production of pigment. In plunge-cultures granular growth, frequently not coherent. Agar-cultures white and not characteristic. No spontaneous movement in a suspended drop.

1. Old G.-cultures a yellowish grey; young cultures white. Margins of the plate-cultures, which are at first translucent and then greyish yellow, are finely scalloped. Great tendency to produce involution forms. Grows even at 0°. Young cultures upon G. containing up to 3 per cent. sodium chloride, agar, salt meat, &c., display in the dark an intense greenish light; if the proportion of salt is small the luminosity is feeble. On continued culture the luminosity may be totally lost. *Bacterium phosphorescens*, Fischer.[1]

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[1] From the further researches of Fischer and Beyerinck we already know six kinds of luminous bacteria, four of which are non-liquefactive (*Photobacterium Pfeigeri, phosphorescens, Fischeri*, and *balticum*, and two liquefactive, *P. indicum* and *luminosum*. For complete diagnoses, and especially for communications on their very interesting behaviour with the different kinds of

2. On old G.-cultures white, never luminous.

a. Upon G. plates a white growth, sometimes dome-shaped and sometimes flat.

α. Surface of the G. plunge-culture slightly elevated, greyish white, rather notched. Short rods joined often by twos, rarely by fours. Spores globular, standing on end (?). Spontaneous movement wanting. Can be stained by Gram's process.

Upon potato a coating 3 to 4 *mm.* in thickness, greyish white, pervaded by small bubbles of gas. Grows best at about 30°. It coagulates milk to a jelly, which then becomes a clot traversed by chinks and a clear serum. Forms lactic acid (of fermentation) from milk-sugar. The most important microbe which turns milk sour. See Hüppe (*Mitth. a. d. Gesundheits-Amt.*, ii.). *Bacillus acidi lactici*, Hüppe.

β. Surface of the G. plunge-culture variable; sometimes foliaceous, strongly extended, sometimes tender and of limited growth. Rods of variable length (1 to 5*μ*). Formation of spores apparently wanting. Decolorised by Gram's method. Forms from milk: lactic acid, butyric acid, formic acid, but no toxines. Flourishes also anaerobically. Upon young potatoes it is a pea-yellow; upon old ones whitish without gas-bubbles. Animals, especially guinea-pigs, die after the injection of large doses (subcutaneous or interveinous) with hæmorrhagic and necrotic processes, especially in the intestinal canal and diarrhœa; swelling of the spleen is not observed. Recently it has been repeatedly recognised as an exciter of dangerous and even fatal infection in man; in other cases it is a secondary intruder. *Bacillus coli communis*, Escherich.

Here follows its nearest ally: *Bacillus neapolitanus*, Emmerich; *Bacillus cavicida*,[1]

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sugar, see Beyerinck. *Over licht voedsel*, &c., Amsterdam, 1890, inserted at length in the *Centralblatt für Bakteriologie*, viii. 616. See also the thorough description of six luminous bacteria by Katz (*Centralblatt für Bakteriologie*, ix. 157). This author has given his species peculiar names, but they chiefly coincide with the species described by Beyerinck.

[1] *Bacillus pyogenes fœtidus* has a certain macroscopic and microscopic resemblance to the former, but all cultures have a putrid odour. In man it produces suppuration, and is fatal to mice and guinea-pigs.

Brieger. See also Smith (*Centralblatt für Bakteriologie*, x. 180).

γ. Opalescent, white, an opaque summit on the G. plunge-culture; also on the G.-plate a white rounded elevation like an upholsterer's nail with a porcelain head ("nail-culture"). Old G.-cultures sometimes show a brownish coloration of the gelatine. On potato a whitish juicy coating interspersed with numerous gas-bubbles. Ferments sugar with production of alcohol, acetic acid, formic acid, and succinic acid. See Frankland (*Centralblatt für Bakteriologie*, x. 222). Under the microscope it appears to consist of very short rods adhering together; when obtained from an animal it is provided with a slight coating capable of being stained. No spores. Decolorised according to Gram. Found in the lungs and the blood in many cases of croupy pneumonia. Pathogenic for mice, guinea-pigs, and dogs on injection into the lungs. See Friedländer (*Fortschritte der Medicin*, i., ii., iii.). *Bacillus pneumoniæ*, Friedländer.

Very similar is *Bacillus lactis aërogenes* (Escherich), from excreta of animals fed on milk; *Bacillus pseudopneumonicus* (Passet), from pus; and *Bacillus of rhinoscleroma* (Paltauf and Eiselsberg); the bacillus of the pulmonary disease of horses (Schütz, Fiedler). *Centralblatt für Bakteriologie*, x. 12.

Compare also *Bacillus* (*Diplococcus*) *pneumoniæ*, A. Fränkel, § 86.

b. On G.-plates minute translucent drops. A moderate but not characteristic extension of the surface layer of the G.-culture; the outline is toothed. The plunge aperture displays unconnected globular masses of microbia. Not characteristic upon agar. The bacilli stain more strongly at the poles than in the middle, thus simulating a diplococcus. Cannot be stained according to Gram. Rabbits, mice, pigeons, fowls are very susceptible. Death occurs in twenty-four to forty-eight hours. See for the bacteria of this section: Bunzel-Federn (*Annalen für Hygiene*, xii. 193); Frosch (*Zeit. f. Hygiene*, ix.); Th. Smith (*Centralblatt für Bakteriologie*, ix. 556). My account is based on the account of the first-named author, from whom other authors deviate only in small matters of detail.

α. No spontaneous movement. They acidify milk and produce indol and phenol.

1. Bacillus of the septicæmia of rabbits, Koch = *Bac. cuniculicida*, Flügge.
2. Bacillus of fowl-cholera, Pasteur = *Bac. choleræ gallinarum*, Flügge.
3. Bacillus of swine-cholera, Löffler, Schütz.
4. Bacillus of game disease, Hüppe. Bacillus of cattle-plague, Kihl.

Nos. 3 and 4 are powerfully pathogenic for swine, and 4 also for deer, horses, and oxen. The infection is introduced also in the breath and in food. According to Hüppe (*Berlin Klinisch. Wochenschrift*, 1885, Nos. 44–46) these four species are identical, the difference of the diseases produced being due to different virulence, different channels of infection, and different adaptation to different animal species. The septicæmia of rabbits and fowl-cholera differ in their cultures (Bunzel-Federn) only by the fact that fowl-cholera can be cultivated upon potatoes, but the septicæmia of rabbits cannot. The Italian buffalo disease (*Barbone dei buffali*) and the "grouse disease" are closely allied. See Klein (*Centralblatt für Bacteriologie*, vi. 2, vii. 3, viii. 2).

β. Spontaneous movement is present. Acidify milk; form indol and phenol.

1. *Marseille swine plague of Rietsch and Tobert.*
2. *Frette disease, Ebert and Schimmelbusch.*
3. *Spontaneous septicæmia in rabbits, Eberth.*

γ. Spontaneous movement present. Turns milk alkaline without precipitation; no formation of indol or phenol. Rapid growth upon gelatine with a brown colour.

1. Hog-cholera, Salmon = swine plague, Billings = American swine disease. The Danish and Swedish swine plague (Lundgren) is identical.

The bacillus isolated by Löffler from the deposits in the throats of diseased poultry and pigeons, *Bacillus diph-*

therœ columbarum (Löffler), is rather longer and narrower than the bacillus of the septicæmia of rabbits.

The growth of the bacilli last.

But slightly characteristic in its manner of growth upon all nutrient media, as is also the case with the septicæmia of rabbits, pigeons, sparrows; rabbits, if inoculated subcutaneously, are attacked with necrotic inflammations. A very characteristic phenomenon is a whitish marbling of the liver in dead mice. The diphtherias of fowls, of calves, and of man are respectively different. L. Pfeiffer regards the microbe as a complication of the flagellated diphtheria; Babes and Pescariu consider it as truly pathogenic (*Zeit. für Hygiene*, viii.).

c. On G.-plates we have translucent, sharply defined, very coarsely granular cultures. The G. plunge-cultures display a luxuriant, greyish white superficial growth, forming afterwards a grey, wrinkled film. Upon A. and P. yellowish grey and luxuriant.

If examined microscopically there are seen short rods, generally connected in pairs. The ends turned towards each other take an intense stain, whilst the opposite ends remain colourless. The size varies, as one to four, according to the nutrient medium (large upon G. and upon A., small in the animal). Spontaneous movement. The cultures, if consumed in food or injected, and whether boiled or not, have a fatal action upon mice, guinea-pigs, rabbits, and fowls, but not upon dogs and cats. It was the cause of the meat-poisoning at Frankenhausen (Gärtner, *Correspondenzblatt des ärtzl. Vereins in Thüringen*, 1888, No. 9), and probably of many other accidents. See Karlinski (*Centralblatt f. Bakteriologie*, vi.), and Gärtner Johnes' recent discovery on meat-poisoning at Cette, *Zeit. für Fleisch und Milch Hygiene*, i. part 9, 1891). *Bacillus enteritidis*, Gärtner.

B. Gelatine cultures produce colouring matter.

a. G. colourless. Colony yellow; [1] short, bulky bacilli, a common aërial microbe. *Bacillus luteus*, Flügge.

[1] For a description of all yellow schizomycetes, see Prowe (*Cohn's Beiträge*, iv. Part 3).

2. Gelatine liquefied.

a. Rapid growth, liquefies G. strongly, very brisk spontaneous movement. Does not take stains by Gram's method. In the blood and the pulmonary tissue, in animals after the vagus has been severed; if injected into the lungs of rabbits causes deadly pneumonia. *Bacillus pneumonicus agilis*, Schou-Flügge.

b. Small, short, spontaneous movement, extremely lively. Nothing characteristic in the plunge-culture. Upon potato a shining, smooth, syrupy covering which rapidly spreads over the entire surface, subsequently slightly wrinkled without deep folds. Frequent on P.-cultures as an impurity derived from the soil. *Bacillus liodermos*, Flügge.

c. Bacillus prodigiosus. See under II.

II. Along with short rods there occur long rods and filamentar forms. Formation of endospores frequent but not universal.

1. Gelatine not liquefied.

A. G.-culture white. G. does not become coloured.

a. Tender nacreous deposit with a toothed margin on the plunge-culture; in the perforation itself a homogeneous, very tender growth. Upon A. a luxuriant, white, succulent growth. A peculiar characteristic is the wide extension of growth upon potato; the microbe forms here an almost invisible, slimy, thin adhesive coating, which draws out in threads if raised up with the needle. Sometimes there occur varieties of the microbe with a distinctly visible growth upon the potato (Ali Cohen, *Centralblatt für Bakteriologie*, iv.), which greatly hinder the diagnosis. Microscopic rods of very different size (regular development of long threads upon potato), lively spontaneous movement, no formation of spores, but with vacuoles and granules which simulate spores (Buchner, *Centralblatt für Bakteriologie*, iv. 353). The bacilli are easily dyed with alcoholic-aqueous aniline colours; rather less readily with carbolic magenta and alkaline methylene blue. Decolorised by Gram's method. Not pathogenic for animals, though some cultures develop a poison (Sirotinin, *Zeit. für Hygiene*, iii.). The literature of this question is to be found in Seitz, *Bakteriologische Studien zur Typhusætiologie*, Munich, 1886. The most recent discoveries are to be found in the *Jahresberichte*. For the physiology, the power of resistance, &c., see Janowski, *Centralblatt für Bakteriologie*, viii. 167 and 417. *Bacillus typhi abdominalis*,[1] Eberth, Klebs, Gaffky.

[1] The diagnosis of the typhus bacilli is peculiarly difficult, as numerous species, not as yet accurately described, behave very similarly both morpho-

Very similar morphologically to the bacillus of typhus is the bacillus of *Purpura hæmorrhagica*, sive *Morbus maculosus Werlhofii*, which reproduces this disease in rabbits, field mice, and dogs. See Babes, *Centralblatt für Bakteriologie*, ix. 719. There can be found here merely a conspectus of the most recent researches.

Similar is also *Bacillus ranicida* (Ernst) in Ziegler's *Beiträge*, viii.; and Sanarelli, *Centralblatt für Bakteriologie*, ix. 193.

β. Superficial growth almost null. From the entire inoculative plunge very minute lines extend radially into the G. or A., so that there arises the delicate

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logically and physiologically. For a differential diagnosis, in addition to the appearance of the potato-culture, we have recourse to spontaneous movement, to the absence of the formation of spores, to the conditions of growth, &c. Certain methods which have been worked out during the last two years may here be tried. Hitherto every author has been dissatisfied with the results of his forerunners, as far as the differential diagnosis of typhus is concerned, and has himself felt the necessity for new improvements. Whether the difficulties that lie in the way will be ultimately cleared up in such a manner that the typhus bacillus is found in a series of somewhat different varieties with fluctuating properties (Babes), whether even the views of Arloing (International Hygiene Congress, London, 1891) will be confirmed, that the virulent typhus bacillus is merely a breed of *Bacillus coli communis*, the next few years must teach us. H. Buchner pronounces the last view as not impossible. It may, however, be very possible that the entire difficulty may arise from the existence of a number of saprophytic species, very similar to the typhus bacillus, though they have absolutely nothing to do with it, but are very difficult to distinguish.

Petruschky has observed the formation of acid and alkali by many species of bacteria in neutralised whey which he had coloured with litmus. It appears that the typhus bacilli are distinguished from similar species by their slight power of forming acids (*Centralblatt für Bakteriologie*, vi. 23, 24; vii. 1, 2).

As a further aid in diagnosing the typhus bacilli from similar forms, Kitasato tests the bacilli for their power of producing indol.

He cultivates for twenty-four hours in 10 *cc.* of broth containing 1 per cent. of peptone, and adds 1 *cc.* of solution of potassium nitrate containing 0·02 *grm.* in 100 *cc.*, and then a few drops of concentrated sulphuric acid. All typhus-like bacteria growing on gelatine (Kitasato tested sixteen species) colour the broth red on account of the presence of indol. Typhus bacteria form no indol, and the broth remains colourless (*Zeit. für Hygiene*, vii.).

Holz, of Löffler's laboratory, has furnished very valuable contributions to the differential diagnosis of the typhus bacilli. He observed that in an acid gelatine not neutralised, which consisted of the expressed juice of fresh, raw potatoes sterilised by boiling, with the sole addition of 10 per cent. of gelatine, the typhus bacilli grew and flourished well, whilst, on the other hand, the majority of other schizomycetes did not grow upon this medium (10 *grm.* require 2·4 to 3·2 *cc.* decinormal lye for neutralisation). By the addition of 0·05 per

figure of a glazier's brush. On microscopic examination there appear the finest small bacilli; the formation of spores has been observed. This is the cause of the bacillar erysipelas of swine and the septicæmia of mice. See Löffler (*Arb. aus d. Kais. Gesundheit Amt.*, i.); Lydtin and Schottelius (*Der Rothlauf der Schweine*, Wiesbaden, 1885). *Bacillus murisepticus*, Flügge, Koch.

γ. Coarse aspect in the entire length of the vaccinated tract (see p. 88). *Proteus Zenkeri Hauser.*

B. G.-culture white or whitish. G. coloured by the diffusion of colouring matter.

a. *a.* G. and A. fluoresce of a greenish yellow; on the

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cent. of carbolic acid to the potato-gelatine the hyphomycetes are very greatly checked in their growth, as also the development of the kinds of bacteria which liquefy potato-gelatine, whilst the development of the typhus bacilli is retarded only by one day. For the examination of samples of soil and dirt we use directly their aqueous infusion mixed with a potato-gelatine containing 0·05 per cent. carbolic acid. In examining waters rich in microbia, we add 0·25 *grm.* phenol to 100 *cc.* of the water in question, and after it has stood for three hours we sow 0·5 to 1 *cc.* in potato-gelatine.

Growth in potato-gelatine is characteristic as follows: the surface-cultures on the plate are very small, scarcely more than 1·5 *mm.* in diameter, and always transparent; those which lie more deeply become darker from day to day, and are at last brownish yellow with a green reflection. In plunge-cultures the absence of a surface growth is remarkable. By means of this method Holz demonstrated the existence of typhus bacilli which had been added to non-sterilised but strongly-contaminated waters fourteen to eighteen days previously. (See *Zeit. für Hygiene*, viii. 237.)

Jäger (*Zeit. für Hygiene*, x. 1891) observed further upon Holz's gelatine, which he had prepared without carbolic acid, the growth in the potato-medium of a bacillus very similar to the typhus bacillus, but yet different. Still he finds the Holz method very useful. Compare also, with reference to Jäger and Holz, a critique of the French proposals to use nutrient media with a larger proportion of carbolic acid, and a decision on the hitherto positive occurrence of typhus bacilli in drinking water. See also Cassedebat (*Annales de l'Institut Pasteur*, 1890). Especially, Uffelman (*Berlin Klinisch. Wochenschrift*) proposed a meat-juice peptone gelatine mixed with citric acid (as much citric acid as will require 14 *cc.* decinormal soda for neutralisation). The gelatine, which is rendered turbid by acidification, does not filter quite clear. To 100 *cc.* there are added 2·5 *mgrm.* methyl-violet dissolved in one drop of absolute alcohol and 1 *cc.* water. After all these ingredients have been sterilised separately and mixed, a small glass is filled, and again sterilised for a quarter of an hour. On this gelatine only very few micro-organisms grow along with the typhus bacilli, which show a fine granulation in the plate-culture, and the colonies become more intensely blue from day to day. Uffelmann used the method with advantage; of course the blue cultures are to be examined as to their behaviour upon all other culture-mediums. Our typhus bacilli in Würzburg unfortunately do not grow upon a medium prepared as above directed.

plate the cultures have a jagged, wavy, bent margin and folds. In the G. plunge-culture moderate deposits on the surface and in the plunge channel. On potato a reddish-brown growth. Slender movable bacilli, often presenting short threads. Very characteristic is the abundant formation of reddish spores. *Bacillus erythrosporus*, Cohn.

β. Similar to the above; fluorescence splendid. Upon potato brownish, bacilli very movable, generally short rods, but sometimes forming thick threads, especially upon potato in the incubation closet. Spores colourless. *Bacillus fluorescens putidus*, Hüppe. *Bac. fluorescens non liquifaciens Autorum.*

b. G. and A. dark brown to blue-black. G. plunge-culture bluish-whitish, shimmering, juicy knobs. Sterile milk remains neutral after infection, but takes a greyish blue colour (the cream first). On the addition of an acid, or the simultaneous presence of lactic bacilli, the colour becomes sky-blue. Potato-cultures are yellowish grey, whilst the potato disc becomes greyish blue. Under the microscope thin bacilli, longer or shorter, and short threads. No formation of spores. Not pathogenic. Bacillus of blue milk = *Bacillus cyanogenus*, Flügge.

2. The gelatine is liquefied.

A. Cultures upon nutrient medium colourless.

a. Upon plates cultures ramified, or provided with offsets; the colonies send off swarms. Large, strong bacilli, which at high temperatures have a great tendency to grow to threads, on which there is an abundant formation of spores. Upon agar nothing characteristic.

α. On the plate a hyphomycetic tangle of wavy threads without a recognisable centre. Growth very rapid. Liquefaction rapid. From the channel there proceed in its entire length a number of stout bristly branches, like the needles of a pine-twig. Spontaneous movement. Upon potato whitish, slimy. Common in earth and air. Root-bacillus = *Bacillus mycoides*, Flügge.

β. Each colony on the plate has a distinct centre.

1. On the cultures present upon the plate a convolution of flocky threads with no very pronounced central point. Pure white. From the upper part of the G. inoculation-channel there proceed short elegant twigs for some distance downwards. In the lower

part they are wanting.[1] Liquefaction is relatively slow without the formation of a film. Upon A. the deposit is coarsely granular, yellowish white. Upon potato greyish white, rather thick deposits, which do not reach a great breadth. The formation of spores takes place only if there is access to oxygen and the temperature of 18° to near 40°, never in the animal body. Easily stained, even according to Gram. The dyed rods have concave contractions at the ends. Spontaneous movement entirely wanting. It can be cultivated only from the bodies of men and other animals, or from the soil of splenic fever localities. Mice, guinea-pigs, rabbits, sheep, oxen, die after the injection of small quantities. The cause of a part of the cases of *Hadernkrankheit*, "Woolsorters' disease." Splenic fever bacillus = *Bacillus anthracis*, Flügge.

2. The plate colonies have roundish centres with a radiating margin.

   a. G. plunge-culture without lateral appendages; the liquefaction soon reaches the margin of the glass, and then proceeds downwards. Finally there floats upon the surface a grey, folded film. Lively spontaneous movement. Potato-culture, yellowish white, extends strongly, and by degrees appears as if powdered with snow-white mealy granules. Very commonly saprophytic. The spores sustain a temperature of 100° for an hour. Previous to the formation of spores the long threads become articulated to bacilli by more distinct furrows. There exists a series of closely allied species; see, *e.g.*, Ludwig Klein (*Centralblatt für Bakteriologie*, vi.), where three are named and accurately described. Hay bacillus = *Bacillus subtilis*, Cohn.

   β. G. plunge-culture resembling the former. Upon potato at first a smooth yellowish deposit; after the second day folded,

[1] But there occur cultures of splenic fever both without branches and with many branches from above downwards. The latter seem to be favoured by higher temperatures.

wrinkled, brown, thin membranes. A common earthy and aërial microbe. "Potato bacillus." *Bacillus mesentericus fuscus*, Flügge. Nearly allied to the alleged "Pellagra microbe," *Bacillus maïdis*, Paltauf and Haider.

b. The cultures on the plate swarm, but only on gelatine containing at most 6 per cent. In form the cultures are extremely various: the flat liquefaction funnels, surrounded by dense garlands of microbes, wander gradually over the gelatine, extending themselves like tongues. In the depths of the gelatine, radiating or clavicular and corkscrew-like outgrowths surround the plate-cultures. The forms of the bacilli are as manifold; all transitions are found, from short, coccus-like rods to long filaments. Endospores are absent. Lively spontaneous movement. Excites the typical putrefaction of meat. It can exist as an anaërobe. See Hauser, *Ueber Fäulnissbakterien*, Leipzig, 1885, and Kuhn, *Archiv für Hygiene*, xiii., which contains the most recent literature. Genus *Proteus*, Hauser.

α. Nutrient media containing albumen or gelatine are very rapidly liquefied with the formation of poisonous and malodorous decomposition products. Common upon putrid meat. Even the sterilised cultures have a poisonous action. *Proteus vulgaris*, Hauser.

β. Similar species are distinguished by a peculiar tendency to produce involution forms, and by liquefying gelatine less rapidly. Perhaps merely varieties of *Proteus vulgaris*. *Proteus mirabilis*, Hauser.

γ. The gelatine remains solid. Numerous stout branches proceed outwards from the plunge-channel; on the surface of the gelatine there is a dense tissue of white threads arranged in a radiating manner. On the plate resembles *Bacillus mycoides*, but more overflowing. *Proteus Zenkeri*, Hauser.

δ. Recently species of Proteus have been recognised as exciting septic processes in man. See Eisenberg's *Bact. Diagnostik*.

c. Colonies on the G.-plate, rounded, without branches.

a. Bacilli 2·5 μ thick, and about four times as long, in general slightly curved. Rapidly forms in G. a liquefaction funnel. Found by De Bary upon stale cabbage. Often studied on account of its unusual size. *Bacillus megatherium*, De Bary.

β. Bacilli not above 1 μ in thickness. Growth upon G. not characteristic; the gelatine becomes a grey, turbid liquid.

a. Upon potatoes; thick white coating, folded like a bowel, extending deeply into the substance of the potato; draws out in threads. It coagulates the caseine of milk like rennet, and afterwards partially re-dissolves it, with formation of slime. *Bacillus mesentericus vulgatus*, Flügge.

b. G. plunge-culture has on its surface a tender folded film. Its favourite temperature is 35° to 40°. Microscopic: large rods, very movable, and growing out into threads. Spindle-shaped before the formation of spores. Caseine is first precipitated, then dissolved to a peptone, NH_3 being split off and a bitter flavour produced. Occasions the butyric fermentation. (For other microbes of butyric fermentation, see § 87.) *Bacillus butyricus*, Hüppe.

B. Gelatine liquefied. Cultures coloured.

1. Formation of red pigment.

a. Almost exclusively oval forms, rare, and only in broth (preferably acid). Forms threads. Spontaneous motion may be either present or wanting. No spores. Keeps well if dried up. Upon G. very rapid liquefaction; colouring matter produced only on the surface; gradually the entire contents of the funnel become rose-colour. On the plate at first circular granulated colonies, then very quickly flat liquefaction funnels with greyish red contents. Upon A. and P. an intense purple red. Older P.-cultures have a greenish metallic reflection like solid magenta. Odour of trimethyl-aniline. Cause of the "bleeding hosts."[1] At very low and very high temperatures it grows colourless, as also in the absence of air. *Bacillus prodigiosus*, Ehrenberg.

b. Short, fine bacilli. Growth as in the former. Optimum temperature 36° instead of 25°. Pigment brick-red, paler. *Bacillus indicus*, Koch.

Allied is the bacillus of red milk described by Grotenfelt (*Fortschritte der Med.*, 1889). The purple pigment is here formed only in the dark. Its growth colours milk red.

[1] Sometimes grows upon bread and other articles made of flour. Occurs sometimes upon the consecrated wafer ("host") in Catholic churches—a phenomenon which in the dark ages caused great alarm, and served as a pretext for massacres of Jews, heretics, witches, &c.—*Translator.*

2. Formation of a green fluorescent pigment. (See Frick, *Virchow's Archiv*, cxvi., "Conspectus of Bacilli with Green Colouring Matter.")
   - *a.* Upon G.-plates round colonies beset with radiating fibres. The plunge-culture soon displays an intense greenish yellow fluorescence, due to the formation of pyocyanine. Yellowish brown upon potato; if a piece of the culture is scraped off, the potato underneath takes a greenish colour, especially if touched with ammonia. Sterilised milk is coloured superficially a yellowish green; the caseine is precipitated, and afterwards peptonised. On microscopic examination there appear small, slender rods, longer or shorter, and often conjoined in groups. Formation of spores frequent. Spontaneous movement. In bluish green pus; the microbe excites suppuration. Bacillus of bluish green pus. *Bacillus pyocyaneus*, Flügge.
   - *β.* Upon G.-plates the colonies have several many-coloured zones with a scolloped margin. Liquefaction very rapid. Plunge-culture at first funnel-shaped, but the upper surface soon becomes totally liquefied. The liquefaction, which is cut off downwards by a turbid sediment, proceeds rapidly. Upon P., brownish. Under the microscope there appear short, movable rods, arranged by twos. Spores have not been found. Extremely frequent in various forms in water, soil, &c. *Bacillus fluorescens liquefaciens*, Flügge.
3. Formation of a violet pigment:

   Grows slowly, and not at high temperatures. Upon G. a violet colouring matter at the apex of the liquefaction funnel. Upon A. and P. violet-black, slender rods, growing to threads; in water. *Bacillus violaceus*, Cohn.

C. Spirilla.

§ 85. Curved rods (comma forms), larger and smaller portions of screws, and finally fully-developed screws, are the sole types of growth. Flügge gives the name of *Spirillum* to everything of this form.

Hitherto only *Spirillum choleræ*, and some species which are readily confounded with it, are of hygienic interest. Other kinds, which have been recently described, can be mentioned only as an appendix.[1]

[1] A classification founded on the formation of endospores or arthrospores is still uncertain. Hüppe proposes to separate:

Without endospores or with arthrospores		*Spirochæta.*
With endospores	Without change in form of cells on growth of spores	*Spirillum.*
	With change in shape of cells on growth of spores	*Vibrio.*

Löffler, in his examination of the flagella, found that the so-called spirilla may be resolved into two groups:

1. True spirilla, with one tuft of flagella at each end of the organisms, which are distinctly screw-shaped. Here belong *Spirillum rubrum*, *concentratum*, and *undula*, the last from water.
2. Curved forms, which might be called comma bacilli, or vibrios, with only one long, serpentine flagellum (or with two bent flagella at one end), as in the mobile bacilli. Here belong the exciters of cholera, and their allies. (*Centralblatt für Bakteriologie*, vi. 209.)

	1. *Spirillum cholerœ asiaticœ* = Comma bacillus (Koch).	2. *Spirillum tyrogenum* (Deneke) = Cheese spirillum.	3. Spirillum of Finkler and Prior = *Vibrio proteus* (Buchner and Gruber), alleged spirillum of *Cholera nostras*.
G. plunge-culture, 20°.	Growth and liquefaction slow at 20°. The small liquefaction-funnel formed appears, after forty-eight hours, chiefly filled with air (by evaporation). In the plunge-channel there is then little growth. The entire contents of the test-glass are liquefied only after some weeks.	Growth and liquefaction more rapid than 1. The latter nearly uniform in the entire course of the inoculation channel. In forty-eight hours the channel is still narrow.	Much more rapid than 1 and 2. Very rapid liquefaction of the gelatine, in the shape of a trouser leg, through the entire channel. G. entirely liquefied in one week.
A. plunge-culture.	Not characteristic. Dirty white.	As in 1.	As in 1.
P. culture.	Brown coating of little extent; developed only at incubation-heat.	No growth at all.	Luxuriant yellowish grey growth at temperature of a dwelling-room.
G. plate, magnified sixty times.	Outline coarsely undulating and toothed. Surface as if strewed with fragments of glass; yellowish brown; halo of liquefaction indicated only in forty-eight hours.	Outline smooth, granulation finer than in 1. Liquefaction more rapid around colonies.	Outline smooth, granulation fine, yellowish. Strong, broad zones of liquefaction, after forty-eight hours, round the cultures; entire plate often liquefied.

	1. *Spirillum choleræ asiaticæ*=Comma bacillus (Koch).	2. *Spirillum tyrogenum* (Deneke) = Cheese spirillum.	3. Spirillum of Finkler and Prior= *Vibrio proteus* (Buchner and Gruber), alleged spirillum of *Cholera nostras*.
Microscopic aspect.	Form of a slightly bent comma most frequent, and S forms rarer. Long elegant screw forms at margin of hanging drops of broth in moist chamber.	In all dimensions rather smaller than 1. Turn of screw narrower, and course lower.	Larger and coarser than 1. Screws shorter.
Spontaneous motion.	Lively shooting movement.	Do.	Do.
Permanent form.	Permanent forms wanting, very sensitive to desiccation. (See Kitasato, *Zeit. f. Hygiene*, v. 134.)	?	?
Staining.	Easy by common methods. Not by Gram.	Do.	Do.
Chemical reaction.	In broth with 1 per cent. peptone in a *pure culture* for twenty-four hours yields indol and nitrous acid by the reduction of nitrates (derived from the gelatine or NaCl). On the addition of purest concentrated sulphuric or hydrochloric acid, a rose-violet colour (Bujwid's reaction).	Bujwid's reaction does not succeed.	Bujwid's reaction gives only a brown red.
Occurrence.	Only in contents of intestines, and in mucous lining of intestines of cholera patients. Not in the blood.[1]	Once in stale cheese.	Obtained by Finkler and Prior in fæces of patients of *Cholera nostras*. Not observed by others. Occasionally elsewhere. In saliva (Miller); in fæces of healthy persons (Kuisl).

[1] For the isolation of cholera bacilli from fæces and other liquids, in which they are not very plentiful, Schottelius proposes firstly to infect a small quantity

	1. *Spirillum choleræ asiaticæ* = Comma bacillus (Koch).	2. *Spirillum tyrogenum* (Deneke) = Cheese spirillum.	3. Spirillum of Finkler and Prior = *Vibrio proteus* (Buchner and Gruber), alleged spirillum of *Cholera nostras*.
Effect.	Guinea-pigs die after injection of cultures into the stomach, after previous neutralisation of the contents of the stomach, and the administration of a little opium. Can be grown anaërobically in eggs: copious formation of ptomaïnes. Hüppe, *Centralblatt für Bakteriologie*, iv.	Like 1, but more feeble.	The same pathogenic action as 1.

The fact that the cholera vibrio occurs in several varieties (Cunningham) is by no means calculated to lessen its importance as a producer of cholera.

Particulars, with figures, are to be found in Riedel, *Die Cholera;* in Koch and Gaffky's *Bericht uber die Thätikgeit der Erforschung der Cholera*, &c.; in the *Arbeit des K. Gesundheits Amtes*, iii.; figures also in Tiemann-Gärtner, *Untersuchung d. s Wassers.*

In all its culture characteristics the *Vibrio Metschnikoffi Gameleia*, the producer of the Russian vibrionic septicæmia of poultry, is very similar to the cholera bacillus. It gives also Bujwid's reaction, though with a rather more yellowish colour. The chief difference is that it is intensely pathogenic for pigeons, in which, after subcutaneous inoculation, it appears in masses in the *blood*. See Pfeiffer, *R. Zeit. für Hygiene*, vii., where there are good photographs.

Kitasato at Berlin has recently described a *Spirillum concentratum*, and Von Esmarch a *Spirillum rubrum*, to which hitherto there attaches no further interest. Both grow slowly, form very long spirals, and do not liquefy G. In *Spirillum rubrum* there are parts which cannot be stained, and represent spores which resist drying up.

Numerous species, curved and spirally twisted, have been described by Waibel (*Centralblatt für Bakteriologie*, ii. and iv.), obtained from nasal mucus, the coating of the tongue, and from the water of swamps.

Sorokin describes a spirillum which he found in a hollow tree. He

with broth in a flask, keep it quiet for twenty hours at the temperature of the body, and then to examine the uppermost layers by the plate method. Gruber, Bujwid, Heine, and Karlinski found this method useful.

shows that its endospores bud out across the longitudinal axis of the mother organism. *Spirillum endoparagogicum* (*Centralblatt für Bakteriologie*, i. 462).

Never yet cultivated, but successfully transferred to apes, is the *Spirochaete Obermeieri* (Virchow), the exciter of *Febris recurrens*. It forms filaments, from 16 to 40 μ in length, in the blood of the patients before and at the beginning of the attack.

II. Conspectus of Aërobic Micrococci and Bacilli which grow only at Temperatures above 20°, and in many cases not at all upon Gelatine.

A. Growth in the Form of Globes, Eggs, and Short Rods.

§ 86. 1. Does not grow upon G. and A. Scanty, tender yellowish green upon blood-serum, which it does not liquefy; its best temperature is 30° to 34°. Consists entirely of roll-shaped diplococci, with a lentil-shaped split. It does not dye by Gram's method, but preferably by Löffler's process. The cocci lodge chiefly in the pus-cells. In the secretion of gonorrhœa and in the pus of gonorrhœal *conjunctivitis* (*Blennorhœa neonatorum*). Has not been transferred to any of the lower animals, but experimentally to men. "Gonococcus." See Bumm, *Mikroorg. der gon. Schleimhauterkrankung.* Wiesbaden, 2nd edition, 1887. *Diplococcus gonorrhœæ*, Neisser, Bumm.

2. Grows only at temperatures exceeding 24°, and then scantily and tenderly, without becoming liquefied even on G. Upon A. and blood-serum, about as tender as the streptococcus of erysipelas. Its optimum temperature is 35°. Rather variable in its microscopic and macroscopic appearance. In contradistinction to the fact that the cultures very easily lose their virulence, and even cease growing if fresh inoculations are not constantly made, the bacilli have in dried sputum a very considerable duration of life and prolongation of virulence, even if exposed to the action of light. Bordoni-Uffreduzzi (*Centralblatt für Bakteriologie*, x. 306.)

Short, oval, microscopic rods, generally arranged two and two, often pointed at their free ends, sometimes arranged in entire chains. Motionless. If obtained from the animal, it is surrounded with a gelatinous capsule which is absent in the culture. Pathogenic for mice, guinea-pigs, rabbits. Most easily to be obtained pure by working up the organs of white mice which have died after the injection of pneumonic sputum (Guttmann). It is probably the cause of the chief part of human cases of pneumonia,

and is also recognised as the cause of empyema, peritonitis, endo- and pericarditis, otitis, meningitis, and cerebro-spinal meningitis. Occasionally found in the mucus of the mouth in healthy persons. Easily stained according to Gram. A. Fränkel's pneumococcus. Micrococcus of sputum-septicæmia. See *Bacillus pneumoniæ* (Friedl., p. 85). Banti, *Centralblatt für Bakteriologie*, ix. 179 and 275. *Diplococcus pneumoniæ* or *D. lanceolatus*, A. Fränkel, Weichselbaum.

On the following species see Kruse, *Centralblatt für Bakteriologie*, vii. 662, and Pansini, *ib.*, ix. 567.

3. In the cells of the exudation in *Meningitis cerebro-spinalis epidemica* there often occur enclosed, according to Weichselbaum, small heaps of a micrococcus which only grows upon A. and serum at the temperature of incubation. It is best stained with Löffler's blue. Decolorised according to Gram (*Fortschritt der Med.*, 1887). *Diplococcus intracellularis meningitidis*, Weichselbaum.[1]

B. Growth as Slender Bacilli.

1. Growing upon A., G.-A., or even G. alone, at temperatures from 25° downwards.[2]
   a. No growth on P., or exceedingly scanty and tardy. On other culture media rather slow, whitish, little characteristic. Best on a mixture of serum and gelatine; fairly well upon glycerine-agar and in milk.

   Microscopic: rather thick, slender rods, the ends often thickened into buttons. No movement. No decided spores, but a great tenacity of life on desiccation.

   Occurs in the deeper layers of diphtheric membranes, once in a healthy child. Pathogenic for guinea-pigs, rabbits, pigeons, *not for mice*. Occasions membranous growths, paralysis, and death. Forms powerful poisons. Never in the internal organs. Roux and Yersin (*Centralblatt für Bakteriologie*, v., vi., and viii.) have confirmed Löffler's communications (*Mitth. des Kais Gesundheits Amtes*, ii. 421). See also Zarniko, *Centralblatt f. Bakteriologie*. Here also, along with the literature of the subject and original observations, we find an account of the very similar though non-pathogenic pseudo-diphtheric bacillus of Von Hofmann, which is distinguished by its broth-culture. *Bacillus diphtheriæ*, Löffler.

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[1] The question of the true exciting cause of *Meningitis cerebrospinalis* is not yet finally decided. Bonome (*Centralblatt für Bakteriologie*, viii.) described an especial streptococcus.

[2] Recently the growth of the bacillus of diphtheria has been repeatedly observed at 20°, on ordinary meat-juice-peptone gelatine.

In the so-called "scarlet diphtheria" the diphtheric bacillus is wanting. See Tange (*Centralblatt für Bakteriologie*, x. 1). Löffler's present point of view is laid down in the *Deutsch. Med. Wochenschrift*, 1890, pp. 5 and 6, and *Centralblatt für Bakteriologie*, viii. 663.

b. Growth upon potato in the incubation-closet is very characteristic; first yellowish, then yellowish brown (colour of honey), lastly dark brown. The A.-culture, and that upon glycerine-agar, whitish, and not characteristic.

Microscopic: small bacilli, slender but strong, very mobile. Formation of spores. Great tendency to develop involution forms.

In the ulcers and nodes of glanders in horses. For the diagnosis we extirpate a swollen lymphatic gland, and institute a P.-culture. Besides horses, field-mice are susceptible (*not house-mice*), also guinea-pigs, rabbits, and men. See Löffler (*Arb. aus dem Kaiser. Gesundheits Amt.*, 1886). *Bacillus mallei*, Löffler and Schütz.

2. No growth upon G. and A. Slow growth upon blood-serum and glycerine-agar at 30° to 34°, but best at 37·5°. On the latter there form thick, concentric cultures of irregular forms. On blood-serum merely small, dry, scaly deposits, which at a low magnifying power consist entirely of S-shaped trains of bacilli. According to Pawlowsky the bacillus grows also on pieces of potato, if they are rubbed with the microbe, and sealed up in glasses to prevent desiccation. No decided formation of spores; sputum which has dried on linen cannot, by any means of preservation, contain reliable bacilli for more than two and a half months (Saritzky).

Microscopic: long, very slender bacilli, motionless, said often to contain spores. On staining, see § 46. Found only in the tuberculous products of man and other animals (pearl disease); produces tuberculosis in all animals experimented upon by whatever method of inoculation. See Koch (*Mitth. K. Gesund. Amt.* ii.). *Bacillus tuberculosis*, Koch.[1]

In general (Maffucci and Koch) we distinguish the bacillus of *human tuberculosis* and that of *birds*. Upon glycerine-agar the former grows dry and scaly, not so luxuriantly as the latter, which forms thick, moist coatings. Hens are said to be not susceptible to human tuberculosis; the dog is to that of birds,

[1] Very similar is the *Bacillus leprœ* (A. Hansen), which occurs in masses in the tissues in leprosy; it also gives the specific stain of the tubercular bacilli, and has been cultivated by Bordoni-Uffreduzzi upon glycerine-gelatine (*Centralblatt f. Bakteriologie*, v.). See also § 46.

The "syphilis bacillus" of Lustgarten probably belongs to this group. Its cultivation has not succeeded; its diagnostic signification is very doubtful in view of the failure of most authors to find it in the tissues of syphilitic patients. See on its coloration, § 46.

less so to human tuberculosis. Rabbits and guinea-pigs are susceptible to both, but the bacillus of bird tuberculosis cannot be permanently acclimatised in them. See "Second Tuberculosis Congress" (*Centralblatt f. Bakteriologie*, x. 298). On the bacillus of pseudo-tuberculosis, see § 249.

III. Conspectus of the Necessarily Anaërobic Schizomycetes.[1]

§ 87. A. *Gelatine not liquefied.*

a. Forms butyric acid and butyl-alcohol from carbo-hydrates;[2] it is turned blue by iodine. Cultures in G. oval, spindle or lemon-shaped, black by transmitted light. *Bacillus amylobacter autorum.*

α. The vegetative cells are cylindrical, straight rods, rarely slightly curved, 0·6 to 0·8μ broad, 3 to 5μ long, often combined in rows; there occur also non-articulated threads. The formation of spores is always in the middle of the cell which assumes a spindle form. The rods when the spores are formed are stained blue by iodine, either in segments or entirely. The cultures when young are nearly globular, light-coloured, and granular. *Bacillus amylobacter I.*, or *Clostridium butyricum I.*, Max Gruber.

β. The vegetative cells are always strongly curved, longer and narrower than in the last-named species. Spores always standing on end; the cell takes the form of a club or a tadpole. Coloured blue with iodine. *Bacillus amylobacter II.*, or *Clostridium butyricum II.*, Max Gruber.

B. *Gelatine liquefied.*

Here belong three important pathogenic species which in their macroscopic behaviour (in plunge-cultures on sugar-gelatine and sugar-agar) can scarcely be distinguished. In the first-mentioned medium they form a greyish, cloudy turbidity with a darker centre; in sugar-agar toothed, fringed bands. Tetanus and charbon symptomatique grow upon both nutrient media more luxuriantly than malignant œdema, and liquefy the gelatine more strongly. All these, in the cultures which contain sugar, generate gas, and

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[1] As the anaërobic plunge-cultures are effected not with a point but a loop, there appears an inoculation chink, not a stab, in which, especially upon sugar-agar, the culture grows as a narrow, jagged band, somewhat fringed. Beside the species here described rather many kinds have been described by Liborius (*Zeit. f. Hygiene*, i.), and Lüderitz (*ib.*, v.), and cultivated upon solid media. Sanfelice (*Centralblatt f. Bakteriologie*, ix.). They are of no practical importance.

[2] See above also *Bacillus butyricus*, Hüppe.

sweetish aromatic, moderately unpleasant odours, which, however, do not allow a diagnosis. On gelatine-plates all three remind us of the hay-bacillus. The chief distinctions are the following:—

	Bacillus tetani, Nikolaier Kitasato.	Bac. of Rauschbrand (*Charbon symptomatique*).	Bacillus of malignant œdema, Koch (*Vibrion septique*).
Microscopic appearance.	Slender rods, and in young cultures often long threads.	Shorter, massive. Ends rounded, rarely threads.	Like *Charbon symptomatique*. Often growing to long threads in the animal.
Spores.	Stand on end; the sporiferous bacillus like a pin.	Spores stand on their middle. Bacilli take a clostridium form; leave the spores free.	Spores stand on their middle. No clostridium form.
Spontaneous movement.	When free from spores, slight movement; when containing spores, motionless.	Lively spontaneous movement.	Lively movement.
Staining.	Stains finely, according to Gram; spores by Hauser's method.	As in tetanus.	Cannot be stained on Gram's method.
Virulence.	Lasting in cultures.	Lasting in A. Not in broth.	Virulence soon ceases.
Locality.	In soil. In animals, only in the pus of the infected place. In inoculation with pure cultures, not to be found at the infected place, even before death. Never in blood and organs.	In bloody serous œdema. Formation of spores only after death, never in blood.	In œdema in peritoneal liquid, forming long threads. Grows only *post mortem*, in organs and blood. Formation of spores after death.
Obtaining.	Tetanic pus from infected point in a spontaneous case is heated to 80° for half an hour, to kill everything but the tetanus spores. Then anaërobic plates. Initial material may be obtained by infecting animals with soil.	From œdema fluid; in spontaneous cases anaërobic plates.	Like *Charbon symptomatique*.

	Bacillus tetani, Nikolaier Kitasato.	Bac. of Rauschbrand (*Charbon symptomatique*).	Bacillus of malignant œdema, Koch (*Vibrion septique*).
Pathogeneity.	Pathogenic for man, mouse, guinea-pig, rabbit, horse. Fowls are not susceptible. Causes *Tetanus neonatorum*.	Non-pathogenic for man, but for cattle (important cattle disease) and guinea-pigs.	Pathogenic for man, oxen, guinea-pigs, mice.

Among these species the bacillus of tetanus has been studied with especial accuracy, particularly their poisonous transformation products, which belong probably in part to the toxalbumens and in part to the ptomaines. Compare Raum, *Zeit. f. Hygiene*, iv.; Kitasato, *ib.* vii. 1889.

Concerning *Charbon symptomatique* see Kitasato, *Zeit. f. Hygiene*, vi. and viii. For various information on the above three microbia I am indebted to my assistant, Dr. Arens, who has for a long time made them the subject of special researches.

IV. Conspectus of certain Schizomycetes from Water which hitherto have not been Cultivated on a Solid Medium.

§ 88. Winogradsky (*Annales de l'Institut Pasteur*, 1890, Nos. 4, 5, and 12, and 1891, No. 2) has recently described, under the name "nitromonas," a small, very interesting ellipsoidal schizomycete, which he regards as the cause of nitrification. This organism, which is sometimes mobile and sometimes quiescent, grows hitherto upon no solid nutrient medium, but is easily raised in a solution of 100 parts lake-water, 1 *grm.* ammonium sulphate, 1 *grm.* potassium phosphate, whilst on the bottom of the vessel is laid 0·5 to 1·0 *grm.* magnesium carbonate. The microbe can evidently build up its protoplasm out of ammonia and carbon dioxide, without the co-operation of chlorophyll or of light. There are formed very considerable quantities of nitrous acid, and also some nitric acid. Although numerous other microbia likewise generate nitric acid, Winogradsky regards his nitromonas as by far the most important. He admits in his last paper that several similar species have to be distinguished. P. and G. Frankland, in England, seem to have isolated the same organism (*Proceedings of the Royal*

Society of London, xlvii. 1890, p. 296). Certain lower algæ are here also appended.

§ 88*a*. Filiform schizomycetes with a distinct contrast of base and apex, which is not the case in all the forms hitherto described. No formation of endospores after the manner of the bacilli. Flügge gives the following arrangement:—

Threads without a gelatinous sheath:—	
Without interspersion of granules of sulphur .	*Leptothrix.*
With interspersion of sulphur	*Beggiatoa.*
Threads with sheath:—	
Not ramified, distinct articulations . . .	*Crenothrix.*
With pseudo-dichotomous ramification and indistinct articulation	*Cladothrix.*

Of the species of so-called *Leptothrix* from the cavity of the mouth Arustamow has grown two pure (*Centralblatt für Bakteriologie*, vi.). Otherwise we know exceedingly little of the morphology and physiology of *Leptothrix*.

Beggiatoa, according to Zopf, consists of long filaments, which at the bottom, where they adhere, are thinner and more distinctly articulated: above they are thicker, and their articulation can be made distinct only by staining. *Beggiatoæ* convert sulphates into sulphides, or into sulphuretted hydrogen, and accumulate sulphur. The best known is the colourless species, *Beggiatoa alba* (Vauch), Trev.[1]

Crenothrix and *Cladothrix* form macroscopic flocks of a pale or dark brown. The latter colour is due to iron oxide, which is deposited in the sheaths. *Crenothrix polyspora* (Cohn) consists of sheathed filaments not ramified, thin below and thicker above, with distinct articulations, extended in length below, but broad and short above. The upper, disc-shaped joints can break up into small parts, which act as spores. The latter either become free or they are developed to threads in the mother-plant. *Cladothrix dichotoma* (Cohn) consists of tender, thinly-sheathed filaments, with an indistinct articulation, often only rendered visible by staining. It is characteristic that in *Cladothrix* an apparent ramification is produced by a lower joint growing laterally past an upper one. Macé (*Centralblatt f. Bakteriologie*, iv.) has recently cultivated this species upon a solid nutrient medium. A grey meadow of microbia with a dry surface. G. and A. take a dark brown colour. G. is liquefied. Both species are not uncommon in water, especially if

[1] *Beggiatoa alba*, commonly known as "sewage-fungus," is not necessarily an indication of animal pollution of a water. It is merely a proof of the presence of soluble sulphur-compounds, and is found, *e.g.*, in certain sulphuretted mineral springs in South-Eastern France, and in the waste waters of chemical works free from excrementitious matter, but containing sodium sulphide. See Slater, "Sewage Treatment and Utilisation."—*Translator.*

impure. *Crenothrix* requires the presence of a small proportion of iron, and the same is alleged concerning *Cladothrix*. Eppinger describes a pathogenic *Cladothrix asteroides* (*Ziegler's Beiträge*, ix.), which occasions a pseudo-tuberculosis in man and in lower animals. See also Almquist (*Zeit. f. Hygiene*, viii.) on certain higher schizomycetes of the genus *Streptothrix*, and the interesting communications of Schmorl (*Hyg. Rundschau*, i.) on the anaërobic *Streptothrix cuniculi*, which is pathogenic for rabbits.

This group of aquatic microbia seems[1] to include the *Actinomyces*, which adhere to the grains of corn, and thus penetrate into the organism, where they occasion tumours and suppuration.

Whilst for the pathological signification of the microbe we must refer to § 249, sentence 13, we must here make the following remarks on its morphology and physiology. In the tumours produced by *Actinomyces* there are found colourless or yellow glands of the size of a grain of millet. They consist of fine hyphæ, intertwined, ramified, and arranged in a radiating manner. They are often forked at the circumference, and are always provided with clavate intumescences. The clubs are produced by the swelling of the sheath around the filaments, and are universally regarded as a phenomenon of degeneration. Boström has first obtained it upon gelatine and blood serum in cultures with reddish yellow centre, and a whitish circumference as if mouldy, among which threads, minute rods, and coccus-like forms are observed. Experiments on the infection of animals have always proved unsuccessful. See Boström in *Ziegler's Beiträge*, ix., part 2. It is said to succeed better anaërobically (Wolff, Twentieth Chirurgencongress, Berlin). Wolff has also succeeded in transferring it to animals. See Bujwid and Protopopoff (*Centralblatt f. Bakteriologie*, vi. and ix.).

V. Appendix to Bacteriological Methodics.

Some Notes on the Higher Fungi.

§ 89. Along with the schizomycetes a number of larger, more highly-organised fungi play an important part in

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[1] Bibliography: Cohn, *Beiträge zur Biologie der Pflanzen*, vol. i. parts 1 to 3; Zopf, *Zur Morphologie der Spaltpflanzen*, Leipzig, 1882; Kubel-Tiemann, *Untersuchung des Wassers*, 3rd edition, 1888–89; Winogradsky, *Ueber Schwefelbacterien Bot. Zeitung*, 1887, Nos. 31 to 37; Winogradsky, *Ueber Eisenbacterien Bot. Zeitung*, 1888, No. 17. Winogradsky gives the name *Beggiatoa* exclusively to species which are *not* attached to solid objects. The sulphur schizomycetes attached he calls *Thiothrix*. He further maintains that the statements of Zopf on the formation of "swarms" are erroneous, multiplication taking place purely by fission. He has described the *Leptothrix ochracea* of Kützing, and describes it as forming sheaths, so that the nomenclature does not agree with the above scheme.

True *Algæ* have also been latterly isolated upon gelatine plates by Beyrinck (*Centralblatt f. Bakteriologie*, viii. 460) by dint of some stratagems.

hygiene, since they set up processes of fermentation or decomposition in articles of food, and are in part also recognised as pathogenic. The fungi here in question (the cap-bearing species do not here concern us) are generally classified from a medical point of view, rather practically than scientifically.

1. *Moulds, Hyphomycetes*, or *Thread-Fungi* (in the widest sense).—These form upon decomposing articles of food a ramified or multicellular mycelium, consisting of soft threads. In many parts there appear fruit-bearers, upon which spores formed non-sexually (conidia) are tied off, or this result takes place with mycelium branches, which are in no respect distinct from the ordinary mycelium. If limited in this manner, forms of development of the most varied classes of fungi (as Brefeld's recent researches have especially shown) fall within the limits of the hyphomycetes. Bearing in mind the difficulty of cultivating the higher fruitful forms, and their often very manifold stages of development, and of interpreting them aright, we must here abstain from entering upon such questions, especially as there are a great number of different species, which can hitherto scarcely be identified according to the descriptions, and which occasionally fall from the air upon our plates and pass for "moulds."

2. *Saccharomycetes, Yeast-Fungi.*—Physicians are accustomed to describe as yeasts all organisms with globular, egg-shaped, or extended cells growing in simple shoots. The botanists have demonstrated that at least the coloured (red or black) ferments do not belong to the genus saccharomycetes, as they never display, like the true species of saccharomycetes, in the interior of the cells a formation of spores (globular, more strongly refractive tissues, often grouped in fours). Most recently Brefeld has decided that all the saccharomycetes belong to the embryology of the higher fungi, and are therefore not independent species. Long ago it has been known that certain forms of development of the moulds (*e.g.*, mucor) have a deceptive resemblance to yeast.

The higher fungi can generally be observed perfectly well

without staining, if it is requisite to examine them apart from the tissues. Yeast can be obtained like the schizomycetes.

For moulds (hyphomycetes) in the tissue, Löffler's process of staining with alkaline methylene blue gives good figures. The hyphomycetes can also be readily dyed by Gram's method.

The majority of the organisms here in question flourish better upon a faintly acid than upon an alkaline nutrient medium. Hence they are often cultivated upon meat-infusion peptone gelatine, slightly acidulated with acetic acid, or upon ordinary gelatine to which has been added a decoction of plums, beer-wort, a decoction of sour bread, or

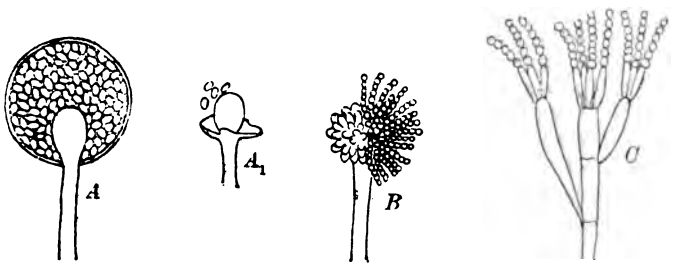

FIG. 38.—Diagrammatic representation of the spore-bearers (conidia) of some hyphomycetes. *A* and A_1 mucor (A_1 represents the columella after the membrane has burst, surrounded by some spores); *B*, Aspergillus; *C*, Penicillium.

the like. The paste is finally spread in a thin layer upon the bottom of a sterilised Erlenmayer flask, and further kept for some hours in the steam-pot. For yeast fungi, beer-wort is the most important liquid medium, and beer-wort gelatine the best solid medium. They succeed well upon sterilised slices of apple.

In planting pure cultures of moulds or yeasts, we proceed exactly according to the rules of plate-culture; a slightly acid medium is used, and the plates are sown as sparingly as possible. The acid reaction prevents at the same time the development of numerous schizomycetes.

If, on the other hand, we wish to isolate schizomycetes

from a mixture which contains simultaneously hyphomycetes, we take a slightly alkaline medium, to which (Schill) we add, before sterilising, a granule of camphor, to hinder the development of the moulds.

VI. Conspectus of some of the most important Genera and Species of Hyphomycetes and Saccharomycetes.

1. Hyphomycetes in the more restricted sense, *i.e.*, Hyphomycetes with Characteristic Conidia.[1]

§ 90. I. Mycelium ramified, articulated. The ascending conidia are often forked at the point; on each point stands a tuft of cylindrical basidia, each of which limits a row of globular, bluish green spores, like a string of beads. Thickness of the filaments 0·7 to 4 μ; diameter of the spores, 3·5 μ. Succeeds best at 26°, not at incubation heat. Liquefies gelatine slowly, producing upon it at first a whitish flocculent, and afterwards a bluish green velvet-like carpet. Everywhere on mouldy objects (Fig. 38, *C*). *Penicillium glaucum L.*

II. Mycelium ramified, filaments articulated. The conidia are generally not ramified, and swell at the point in the shape of a bladder. On the outer surface of this bladder there stand stellated sterigmata, simple or divided, each of which demarcates a chain of spores (conidia). If the access of air is restricted, the genera belonging to this group form permanent fruits which cannot be considered here.

1. Sterigmata ramified (sub-genus, *Sterigmatocystis*); permanent fruits massive.

A. Carpet a chlorine green; when older a brownish, dirty green. Conidia partly ramified brownish red; spores 3 to 4 μ in diameter, yellowish green; optimum temperature, 40°; pathogenic for animals. *Aspergillus nidulans*, Eidam.

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[1] This group, which is regarded by the lay public as unconditionally closely connected, consists—as cannot here be shown in detail—of genera which are widely remote from each other. The higher fructification which these fungi sometimes form places penicillium along with the tuberaceæ (truffles), aspergillus with the ascomycetes, whilst mucor, in which conjugation has been demonstrated, belongs to the zygosporeæ.

Details on the above and other forms of moulds may be found in Rabenhorst-Winter, *Kryptogamen Flora Deutschlands*, 2nd edition, section "Fungi;" Siebenmann, *Die Schimmelmykosen des menschlichen Ohres*, Wiesbaden, 1889; Lichtheim, *Path. Schimmelpilze*, B. kl. W., 1882; Lichtheim, *Ueber pathogene Mucorineen* (*Zeit. f. Klinische Med.*, vii. 2); Lindt, *Ueber einige Path. Schimmelpilze* (*Arch. f. Experimentelle Path. und Pharm.*, 1886); Brefeld, *Botan. Untersuch. über Schimmel- und Hefepilze*, Parts 1 to 8.

B. Carpet black or dark brown, 3·5 to 4 μ; optimum, 34°. *Aspergillus niger*, Van Tieghem.

Here belong *A. ochraceus*, *albus*, &c.

2. Sterigmata not ramified; permanent fruits massive. *Aspergillus* in the more restricted acceptation (Fig. 38, *B*).

A. Carpet bluish green, becoming afterwards a dirty grey. Entire plant small, spores small, round, in general colourless, 2·5 to 3 μ; optimum, 37° to 40°; minimum, 15°; pathogenic for animals; attaches to the heart, kidneys, and labyrinth; disturbances of equilibrium. *Aspergillus fumigatus*, Lichtheim.[1]

B. Carpet yellowish green. Smaller than *A. glaucus;* larger than *A. fumigatus*. Spores yellow or brown; surface finely warted 5 to 7 μ; optimum at 28°; pathogenic. *A. flavescens*, Wreden = *A. flavus*, Brefeld.

3. Sterigmata not ramified; permanent fruits with tender skin. Genus *Eurotium*, De Bary.

A. Carpet blue to yellowish green. Spores oval or globular; yellowish green; diameter, 9·15 μ; sometimes smooth, or sometimes with fine gibbosities; grows well at 10° to 15°, not above 25°; not pathogenic; occurs very widely. *Eurotium Aspergillus glaucus*, De Bary.

B. Similar with smaller spores (4 to 5½ μ); mycelium spreading far; growth scanty at 30°; not pathogenic; very common. *Eurotium repens*, De Bary.

III. Mycelium ramified, undivided, stronger than in the species of *Aspergillus;* the conidia are ramified or not ramified. The spores are formed in a rounded terminal bladder (sporangium) of the fruit-bearer, into which the bottom is inserted in the form of a dome (Columella, Fig. 38, *A*). After removing the outer membrane of the bladder, the columella covered with conidia presents figures which, on hasty examination, remind us of the *Aspergilli*, but the sterigmata are of course absent (Fig. 38, A_1).

In many species the conjugation of two branches of the mycelium has been observed. If oxygen is wanting *Mucor racemosus* forms in sugary liquids yeast-like growths which possess a fermentative action. *Mucor*.

1. The supporters of sporangia not ramified, save exceptionally.

[1] *Aspergillus fumigatus* occasionally produces in France the disease of the pigeon-stuffers which progresses like tuberculosis (*L'Union Medicale*, 1891, No. 38); and Chantemesse (*Centralblatt f. Bakteriologie*, vii. 775).

A. Conidia very long. Sporangia yellowish brown to black, smooth or beset with spines of calcium oxalate; spores oval brownish, 8 μ in length and 4 μ in breadth; everywhere. *Mucor mucedo L.*

B. Mycelium curved and spreading far over, sending out a hair-like root at the points of contact with the nutrient medium. Sporangium black, spores brownish, almost globular, from 10 to 20μ. *Mucor stolonifer.*

C. Similar, but smaller in all parts, the supporters of the conidia always plural; the mycelium has a root where they take their origin; columella ovoid; sporangia dark; spores colourless 5 to 6 μ; pathogenic. *Mucor rhizopodiformis*, Lichtheim.

D. Rather similar to the above, but spores smaller; slight, oval, 2·5 to 4 μ; found in the auditory passage. *Mucor septatus*, Bezold.

E. Mycelium tender, low; aërial mycelium almost absent. Supporters of sporangia simply ramified; sporangia slightly spinous. Spores very small, round, 3 to 3·5 μ, colourless. Optimum, 45°; not below 25°. Cannot be stained with the ordinary solutions. Pathogenic. *Mucor pusillus*, Lindt.

2. Supporters of sporangia branching or tufted.

A. Mycelium whitish-grey; sporangia colourless, pear-shaped, distinctly bounded, attached to the extended sporangial supporter. Spores colourless, longish, 3 μ long, 2 μ broad. *Mucor corymbifer*, Lichtheim.

Very similar to above, but sporangia rather darker. Spores larger, 3 μ to 4 μ broad and 5 μ or 6 μ long. *Mucor ramosus.*

2. Pathogenic Hyphomycetes without the Mould Character.

§ 91. Here approximates a group of hyphomycetes which have only been examined of late, and are still little known in their botanical relations. They have no special conidia, and their non-characteristic behaviour either requires a detailed description with illustrations or compels us to be content with brief indications.

I. *Achorion Schönleini.* The fungus of favus. According to Grawitz: Distinctly articulated hyphæ cohere to a mycelium abundantly ramified, the branches of which often strike off at right angles,

and are peculiarly wound. It flourishes only in the incubation closet, and even there but slowly. Upon serum there are formed elliptical conidia without special supporters. Various investigators differ considerably from each other in their accounts of the formation of the spores, sporangia, &c. Upon G. there is slow growth with gradual liquefaction, first as a whitish flocky layer and then thick, dry, and white. The lower side of the carpet is yellow. Grows also upon agar and potato.

Quinke's *a* fungus, said to occasion *Favus herpeticus*, differs from this species, which Quinke recognised as the cause of *Favus vulgaris*.

Favus and *Herpes* have been transferred by Grawitz from pure cultures to human subjects; other authors have in part met with only negative results.

II. *Trichophyton tonsurans.* Fungus of *Herpes tonsurans.* According to Grawitz this fungus is very like the above, but the filaments

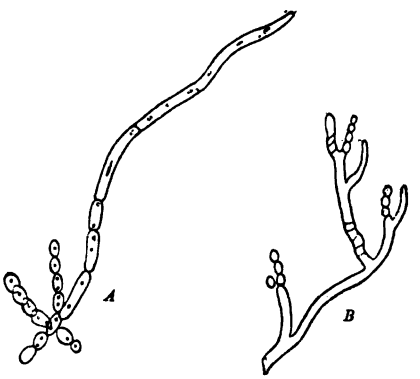

FIG. 39.—*A, Monilia candida; B, Trichophyton tonsurans* (diagrammatic).

are more rectilinear; the conidia are produced in rows like a German roll (*semmel*) (Fig. 39, *B*). Requires an incubation temperature, and an alkaline reaction; liquefies G.; succeeds on agar and serum, not on potato. Grows still more slowly than achorion.

III. *Oidium albicans*, the fungus of aphthæ (plant) = *Monilia candida* (Bonorden), very common in stables, dung, milk, on moist wood, &c.

Upon saccharine cultures, poor in water and cut off from air; this fungus grows like yeast, and excites fermentation. Upon nutrient media, rich in nitrogen and water, it forms articulated filaments, longer or shorter, which in many places support rounded or oval conidia 5 μ to 6 μ long, 4 μ broad (Fig. 39, *A*).

Upon G.-plates there are developed partly round, coarsely granulated cultures, reminding us of yeast-colonies, and partly cultures with radiating, ramified processes.

G. plunge-cultures display whitish threads on all sides of the channel in its entire length ; on the surface of the G. a yeast-like growth ; G. not liquefied. Pathogenic for poultry and pigeons on inoculation in the crop, *i.e.*, developing characteristic aphthous membranes.

IV. *Oidium lactis* is an often abundant inhabitant of sour milk. It is said to have no influence upon the milk. Pathogenic properties apparently absent. Its relation to *Oidium albicans* is not perfectly clear to me.

V. *Mycoderma vini* and *Mycoderma cerevisiæ* form upon wine and beer dull grey mould-films, often wrinkled ; nearly related to *Oidium albicans*, to the achorion, and the trichophyon. From the discordant descriptions given by authors it would seem as if young, imperfectly developed forms of different species of higher fungi have been described under this name.

The new literature of this subject which constantly appears contributes a number of discordant statements, from which any one who does not specially occupy himself with the dermatomycetes can only conclude that there appear an entire series of imperfectly characterised fungi, or dermatomycetes. But to determine which of them is the most important I am at present unable to decide, and I must confess *Non liquet.*

For nearer explanations see—

Favus and *Herpes tonsurans:* Virchow's *Archiv*, B. 103 ; Quinke, *Archiv f. Experim. Pathologie*, xx., and *Monatshefte f. Prakt. Dermatol.* 1887, No. 22, and 1889, No. 2 ; Pick and Kral, *Untersuchungen ueber Favus, Beiträge z. Dermatol. und Syphilis*, i., Vienna, 1891 ; Frank, L., *Monatshefte f. Prakt. Dermatol.*, 1891, vi. ; Unna, *Flora dermatologica*, *Monatshefte f. Prakt. Dermatol.* since 1889

Munnich, *Archiv f. Hygiene*, viii. ; Verjuski, *Ann. de l'Institut. Pasteur*, 1887 ; Elsenberg, *Archiv f. Dermatol. und Syphilis*, 1889.

Soor (*Oidium albicans*) : Grawitz, *loc cit.* ; Hugo Plaut., *Beyträge zur Syst. Stellung des Soorpilzes*, Leipzig, 1885, and *Neue Beyträge*, &c., Leipzig, 1887 ; Laurent, *Centralb att f. Bakteriologie*, viii. 407.

3. Yeast-Fungi, Saccharomycetes.

§ 92. The better-known yeasts are so similar in their forms of growth that without cultures conducted exactly according to the directions of recent investigators a diagnosis of the several closely related species is not possible. The diagnosis depends especially on the following points, which must be carefully determined :—

1. Limits of temperature within which the formation of ascospores takes place upon moist, sterilised blocks of gypsum.

2. Forms of cells which appear in the films formed upon beer-wort; the films are preferably produced at 13° to 15°.

The following conspectus of the six true species of saccharomycetes of Hansen, described by Jörgensen (*Mikro-organismen der Gährungsindustrie*, Berlin, 2nd edition, 1890), is intended merely to allow of a general view of the degree of difference and the physiological significance of the species. Useful work in this region presupposes special study in an institution for the industry of fermentation.

By the recent researches of Hansen (*Zeit. f. d. gesammte Brauwesen*, 1890, 145) the difficulty of this subject has been further increased, since it is shown that the property of forming spores, as in the schizomycetes, may be lost by culture, and the property of forming films in the varieties of culture may be greatly modified.

Saccharomyces.	Limits of Temperature for Formation of Spores.	Forms of Cells in Film produced at 13° to 15°.	Properties.
Cerevisiæ I.	11° to 37°	Chiefly rounded and round-oval forms.	Typical yeast of top fermentation; works violently at 14° to 18°; bottom-yeast which works at 4° to 10° is merely a variety.
Pastorianus I.	3° to 30½°	Commonly prolonged sausage-like cells, resembling mycelium.	Occasions a bitter taste in beer-wort.
Pastorianus II.	3° to 28°	Predominant oval and round.	Causes no disease in beer.
Pastorianus III.	8½° to 28°	Like Pastorianus I.	Causes yeast-turbidity.
Ellipsoideus I.	7½° to 31½°	Richly ramified mycelium; cells thinner and thicker; branches often fixed like a "quirl" (chocolate stirrer).	Yeast of grapes and of the fermentation of wine.
Ellipsoideus II.	8° to 34°	Oval-roundish.	Causes yeast-turbidity.

Similarly different species were found by Marx in examining the lyes of grapes and raisins. On the yeasts which ferment lactose (without spores), various points have been recently determined; the entire literature of the subject may be found in Beyerinck (*Centralblatt f. Bakteriologie*, vi.).

Schwanhauser (*Centralblatt f. Bakteriologie*, ix. 101) has recently succeeded in demonstrating further differences among the yeasts. He compares the two kinds considered as *Saccharomyces cerevisiæ* with *Saccharomyces Pastorianus III.*, and on cultivation upon plum-gelatine, beer-wort (not hopped), beer-wort gelatine, and potato he found as regards luxuriance, juiciness, configuration of the culture, liquefaction of the nutrient medium, odour, &c., the widest differences.

Along with the true species of saccharomycetes certain organisms of kindred types of growth, but without formation of spores and of very uncertain botanical position, play an important part in the brewing industries (see Mycoderma, § 91).

Saccharomyces apiculatus. Elliptically rhombic cells with buttons at the extremities (lemon-shape), and along with them all possible types, but always without spores. Cannot eliminate invertine or ferment maltose, but only dextrose. It is, according to Martinand and Rietsch (*Comptes Rendus*, cxii. 736), a prominent agent in the fermentation of wine. For further particulars compare *Zeit. f. Hygiene*, x., "The Morphology and Physiology of the Yeast-fungi."

VII. Second Appendix to Bacteriological Methodics.

Some Foundations for the Search for Parasitic Protozoa.

§ 93. Along with the vegetable parasites above described, and the larger animal intruders to be discussed in the chapter on meat, we have in the course of years become acquainted with a series of small pathogenic animals not sufficiently known from a zoological point of view. They belong to the group of the lowest protozoa.

For more minute expositions in this difficult and little known regions, we must in the first place recommend L. Pfeiffer, *Die Protozoen als Krankheitserreger*, 2nd edition, 216 pages, 91 figures, Jena, 1891, and his separate papers in the *Zeit. f. Hygiene*, iii., iv., v., vii., and viii., where the author has collated the methods of investigation. Compare further M. Braun, *Bericht über die Fortschritte in der thierischen Parasitenkunde* (*Centralblatt f. Bakteriologie*, x. 389), with critical references to the most recent literature of the subject. L.

Pfeiffer, in *Centralblatt f. Bakteriologie*, viii. 761, gives a clear preliminary introduction to the difficult systematics of the subject. In the subjoined I have chiefly followed the expositions of Pfeiffer in the following descriptions. Unfortunately the methods of investigation are still extremely imperfect; the forms of the organisms in question are scarcely distinguishable from leukocytes, cell-nuclei, and flakey, necrotic tissue, and specific stains are not known. We do not here enter into classification of the protozoa, with its copious nomenclature, the observation of the living organisms which move in the manner of amœbæ or by means of flagella upon heated object-stands, and their fixation with the fumes of osmic acid for preservation are hitherto the chief auxiliary means of investigation. Little has hitherto been effected with staining; osmic acid is said to do good service (Neisser, *Molluscum contagiosum*, *Vierteljahr. f. Dermatol. und Syphilis*, 1888, iv.). The reference of the most important forms to given zoological groups is scarcely determined.

Hitherto the significance of these organisms has been shown chiefly for some of the diseases of the lower animals. Some instances of such affections are:—

1. In the pébrine of the silk-worm, spores of a dull lustre, and having hard shells (Cornallia's corpuscules), are devoured by the young caterpillars. From them emerge in the intestines amœboid moving beings, which then, being in part carried away by penetration into the blood-corpuscules, become larger, more sluggish masses with a double contour (pecto-plasmatic stadium), in which again there are formed the Cornallian corpuscules, soft at first, and afterwards assuming hard shells, and set free by the decay of the parent. The organisms are characterised as microsporidia.

2. In tubes between the muscular fibres of different animals (Miescher's tubes), and in capsules with tough sides hanging loosely to the oesophagus especially of sheep and goats, there are formed similar organisms. In the larger capsules there are formed tubes of the second and third rank one within another. The rare youngest stages represent large pectoplasmatic organisms with a double contour,

instead of which we more often find their derivatives, semilunar organisms with peculiar movements. From the half moons, which are considered as the typical contents of the tubes, there proceed nucleated forms resembling Gregarines, which on the addition of an acid emit two filaments from one end of the body. The transfer of these structures to other warm-blooded animals by inoculation, or in their food, has hitherto proved impracticable. They seem to enter the animals by the consumption of those stages of development which live in snails. In man isolated cases only have been observed, which possibly may depend on such infection; they terminate fatally with the aspect of a parenchymatic polymyositis, as in an infection of trichinæ (see Unverricht, *Münch. Med. Wochenschrift*, 1887, No. 26). How in such cases the parasites enter into the human system is doubtful, though Rabe (*Adam's Wochenschrift*, xx. 167) states that he has observed a case of flesh-poisoning after the consumption of pork containing psorospermia. It was attended with violent catarrh of the stomach and bowels, but there was here probably a complicated cause (sepsis, &c.) Hitherto Virchow's view is still valid (*Virchow's Archiv*, xxxvii. 1886): "Hitherto no case has become known where the consumption of pork containing psorospermian tubes has had injurious effects upon man." These organisms are provisionally named sarcosporidia.

3. By coccidia we understand a large group of parasites which live parasitically within the cells, and which have hitherto been observed in rabbits (*Coccidium oviforme*, Leuckart), where the full-grown parasite is 35 μ long and 15 μ broad; and also quite similar forms in calves, dogs, sheep, and in birds. Their entire development takes place in the epithelium cells of the liver and bowel. On coccidia in the human liver, see Podwyssozki (*Centralblatt f. Bakteriologie*, vi.).

According to the discovery of Richard Pfeiffer, further worked out by L. Pfeiffer, there occur at least in the prototype of the coccidia, *C. oviforme*, two methods of multiplication. The form which has been earlier known is this that

the young amœboid parasite penetrates into an epithelial cell of the bowel or the liver, and when fully grown encloses itself in a capsule. Within the sporogonia (sporoblast) there are formed after their expulsion four sporocytes, each of which contains two falciform bodies. It seems that by the consumption of such matured sporoblasts new infections can be determined, but which always have a chronic character of but little malignity. Such is the process in older animals.

In younger subjects there arise, from the amœboid falciform germs which penetrate into the cells when they have reached full growth, bladders enclosed in a thin skin, in which there develop numerous daughter-cells, at first of a rounded shape, each of which becomes a falciform body.

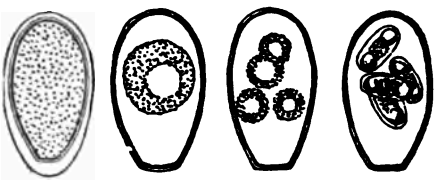

FIG. 39*a*.—*Coccidium oviforme* from the liver of the rabbit, according to Leuckart. 1:600.

1, Coccidium in an epithelial cell of the biliary duct; 2, coccidium free in a capsule, protoplasm contracted to a globular form; 3 and 4, origin of four pseudo-novicellæ; in 4 may be seen the C-shaped germinal rods.

The falciform cyst bursts, the falciform bodies become free amœboids, penetrate at once into new cells, and begin afresh their destructive activity. Thus there arises a very dangerous progressive disease. Young rabbits may be easily infected with ripe sporoblasts. Fig. 39*a* shows the old manner of development of *C. oviforme*. Fig 39*a* represents the propagation of a parasite of the mouse according to the second type. Schuhberg has recently shown that this organism also, which has been described as a special genus, Eimeria, increases, under certain circumstances, according to the first type.

4. The contagious epithelioma of poultry and pigeons is occasioned by similar organisms, whilst the *Molluscum con-*

tagiosum of man (Neisser) is due to the same or to similar parasites.

5. More highly differentiated are the forms (genus *Trichomonas*) which determine the flagellate diphtheria of birds (poultry and pigeons). The interesting announcements made on this subject by Rivolta in 1869 have been extended and rendered more precise by L. Pfeiffer. The unitary character of the diphtheritic processes in birds is brought into prominence. Löffler's bacilli of the diphtheria of pigeons (see p. 80) were also detected by Pfeiffer, but he regards them as a septic complication. At the summit of their development the organisms are oval or semi-lunar, but in any case very variable, with four flagella at the head, a

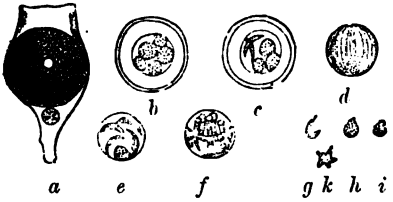

FIG. 39*b*.—Coccidium from bowel of mouse (Eimeria), according to Leuckart.

a, Naked coccidium in an epithelial cell of the bowel; *b*, *c*, the same with a capsule; contents in their change into falciform bodies, which are liberated in *g*, and display amœboid movement, *h* to *k*.

divided one at the tail, and an undulating marginal membrane. There are also vacuoles, one of which is contractile. On the action of reagents the flagella are drawn in or thrown off, and there appear forms which cannot be distinguished from the white blood-corpuscules. From the flagellate state these organisms pass, by losing their lashes, into an amœbic condition of great contractility; but there may also occur a state of perfect rest. Finally incystment and the formation of spores, after the manner of the pébrine organism, may ensue. The affections produced in birds by Nos. 4 and 5 are connected by transitions.

§ 94. Allied to the forms described are those recently detected (Laveran, 1880), since verified by numerous investi-

gators, and further studied as exciters of malaria, which are now universally recognised. The only contest now is if all the parasites observed are to be regarded as varieties of one species, which Marchiafava and Celli (to whom we are indebted for numerous researches in this region) term *Plasmodium malariæ*, or if, like Golgi, Canalis, Grassi, Feletti and others, we must distinguish several exciters of malaria.

The following character is common to all the forms: the young, very small parasite, according to Grassi and Feletti, consists of nucleus and ectoplasm; it penetrates into a red blood-globule, and grows therein with spontaneous movements of an amœboid kind nearly to its full size; then there ensues a division of the parasitic nucleus; around the nuclei is arranged some of the ectoplasm, and the parts (spores) are set free as young amœbæ.

All the forms may occur pigmented (Melanine) or free from pigment. The parasites are easily seen in motion on the heated object-stand. The figures are so far the clearest. A prick into the point of the finger yields a minute blood-drop, which is quickly spread out between the object-slide, previously flambé, and the covering-glass is examined. The parasites are easily stained with methylene blue; double staining, by dyeing the blood-corpuscules with eosine, is also not difficult. Sections of the brain and spleen yield

FIG. 40.—*Plasmodium malariæ* (most closely resembling the quartan type).

likewise very good figures. Super-inoculation of melanic blood upon the healthy blood yields malaria with the occurrence of the form of malarial plasmodia which were present in the primary case. This is a main support for the hypothesis of several forms.

The separation into three forms is founded on the following characteristics:—

1. Parasites of the quartan and tertian ague in their various forms, or parasites of the normal European

malaria forms. In no stage does the parasite present semi-lunar forms or flagelliform processes. Genus, *Hæmamœba* (Grassi and Feletti).

According to the careful studies of Golgi (*Archivio per le Scienze Mediche*, x. and xiii.), of late repeatedly confirmed, it is found that the sporulation and the emigration of the young amœbæ into new blood-globules precedes the attack of fever by a short time. We distinguish:

a. The *parasite of tertian fever.* It shows in the blood-corpuscule thin, often ramified offsets and decolorises the blood-corpuscule when it has completed its growth; forms numerous spores.

b. Parasite of quartan fever. Offsets short and thick: when at the height of its development it fills up the entire blood-corpuscule; number of spores small, spores large, forming for a time the figure of a daisy. Golgi has published excellent photographs (*Zeit. f. Hygiene*, x. 1891).

2. *Parasites of irregular fevers.* When at the height of its development the parasite frequently or regularly takes the shape of a half-moon, or kidney. Peculiar filaments are observed, which, however, are variously interpreted (*e.g.*, by some as flagella, by others as pseudopods, and by others even as merely as phenomena of mortification). Particularly studied by Laveran and Canalis. Genus, *Laverania* (Grassi and Feletti).

For the bibliography of the subject see Laveran, *Du Paludisme et de son Hématozoaire*, Paris, 1891, and the very numerous memoirs and references in *Centralblatt f. Bakteriologie.* In the same journal are to be found new accounts of the parasites of the blood of birds, first studied by Danilewsky, and standing zoologically very near to the organisms of malaria. See also Celli and Sanfelice, *Fortschritte der Medicin*, 1891, No. 12. The parasites of birds, however, do not seem

to have any genetic connection with the exciters of human malaria. Transferences from one to the other have not been found practicable, although Danilewsky regards them as identical. All attempts at the artificial cultivation of malaria-organisms, or to find them in swampy soils outside of man, are hitherto without demonstrative results. See Grassi and Feletti, *Centralblatt f. Bakteriologie*, vii. 396.

Lastly, dysentery, and at least a part of the abscesses of the liver, may be certainly traced to a kind of amœba discovered by Lösch, and named *Amœba coli*. This parasite has been especially studied by Kartulis in Egypt in the last year, and has still more recently been cultivated in infusion of hay (*Centralblatt f. Bakteriologie*, ix. 366). All authors in different parts of the world confirm the statements. For a conspectus of the literature see Dock (*Centralblatt f. Bakteriologie*, x. 227). At the same time amœbæ may be found in small number in the intestines of persons in health. Whether these organisms are identical with the amœbæ of dysentery is quite unknown.

It is probable that in the coming years, especially if more perfect means of investigation are discovered, protozoa will in many cases be found to be the cause of infectious diseases which have been hitherto a riddle. The researches of L. Pfeiffer have made this very probable; for the group Variola, Vaccine, Ovine, Varicellæ, and also for Apthæ and Cattle-plague (Steppe-fever), there are positive indications. In a series of malignant tumours Pfeiffer and others have detected protozoa. Even the organisms found by Klebs in struma may rank here. See also Plehn's *Aetiologie und klinische Malariastudien*, Berlin, 1890; and Spener, *Zusammenfassender Bericht über den Erreyer der Malaria* (*Biolog. Centralblatt*, xi. No. 12).

NOTES BY TRANSLATOR.

The Rev. Dr. Dallinger, F.R.S., has shown that by carefully managed cultures the temperature at which many species of protophyta flourish can be greatly modified. Hence the value of the optimum temperature in diagnosis is often rendered doubtful.

The larger and higher animal parasites, *e.g.*, trichinæ, tœniada, trematoda, &c., are mentioned by the author under the Examination of Meat.

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SECTION III.

SOME INDICATIONS ON HYGIENIC-TOXICOLOGICAL INVESTIGATIONS.

§ 95. Kobert, in agreement with Husemann, gives the following general definition of the idea of poison, which is satisfactory in view of the difficulty of the question :—

"Poisons are substances, partly inorganic, partly organic (but non-organised), whether obtained artificially or occurring in nature, which, in virtue of their chemical character, can, under certain circumstances, so affect any organ of living beings, that the health or the relative well-being of the organisms is seriously affected, whether temporarily or permanently."

From the hygienic point of view a substance is a poison when it, if employed in the manner and in the doses which occur or may occur in practical life, and on shorter or longer use, occasions subjective or objective derangements of animal (especially human) well-being. Substances which injure mechanically (pins, fish-bones, &c.) are not included among poisons,[1] nor are organisms.

As regards the toxicity of most of the substances with which hygiene is concerned, the material at our disposal for reaching a decision is insufficient. While we are perfectly certain as to the toxicity or the harmlessness of large doses of many substances—especially inorganic—it is very difficult to say if, *e.g.*, minimal quantities of tin, zinc, or nickel, if taken daily for a length of time, prove injurious; whether health is imperilled, or may in occasional cases be imperilled, by the constant ingestion of small quantities of salicylic acid, by the inspiration of sewage gases, &c.

[1] It is in some cases an open question whether the injuries occasioned by the hairs of certain caterpillars are of a chemical or a mechanical character, or if they may not act in both ways.—*Translator.*

The decision of such cases is no less important than difficult. The experiments must in strictness also be performed upon men,[1] the form of the introduction must be that which occurs in nature. The persons submitted to experiment must during the very tedious trials be protected from all other derangements of health, and lead an absolutely regular life. That these conditions are not easily satisfied is manifest. If neither the investigator himself, nor any person who is willing and whose actions can be accurately controlled, is prepared for the chronic experiments, the following expedients may be employed as substitutes:—

1. Shorter experiments on man with rather larger doses. This is practicable only if severe injury is not to be expected. It is, *e.g.*, admissible in case of salicylic acid, boric acid, fusel oil, nickel salts, but not with lead chromate, corn-cockle, &c.

2. Dietetic experiments on different animals, if possible: white rats, rabbits, dogs (or cats, which are cheaper and take up less room), and one species of bird, say poultry or pigeons. Such prolonged feeding experiments require great conscientiousness, cleanliness, and care, and, above all, a suitable room capable of being warmed, as the observations will be of value only if made upon subjects kept in an unobjectionable state. If possible the probang should be avoided, the substance being mixed with food which is voluntarily eaten, but the consumption of which is under control. Each animal requires a special cage, kept carefully clean, and placed on a support (§ 77). If the substance to be consumed has a bad taste it may be made acceptable to the phytophaga by wrapping in lettuce leaves, insertion in carrots, &c. Carnivorous animals may be induced to eat by an addition of extract of meat. In each case repeated experiments may be necessary.[2]

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[1] In Britain man is the only animal upon which it is legally permissible to experiment without formalities, which are always difficult and often altogether impracticable.—*Translator.*

[2] It is scarcely necessary to add that these experiments can only be performed safely in Britain under license from the Home Secretary. Or the investigator must take up his abode in France or Germany.—*Translator.*

As a supplement to, but never as a substitute for, dietetic experiments, subcutaneous injections may be applied, always keeping in view the possibility of an infection. If, *e.g.*, any one proves the injurious character of a water by subcutaneous injection, this is certainly an unhappy form of demonstration, even though the fact in conjunction with others may be very interesting.

It is still more erroneous to effect injections into blood-vessels for any purpose save for further physiological-toxicological explanation of the *modus operandi* of the substance in question. Here also there is the risk of undesigned or even perfectly accidental injury, solvent action upon the blood-corpuscules, production of coagulations in the blood which may occasion embolisms in vital organisms, direct action upon the heart, &c.

The injections are effected exactly as described in the technics of bacteriology. There is, however, one case in which they may render excellent service to the experienced operator, *i.e.*, for the first clue to the toxicity of a substance which has been obtained only in small quantity, since nearly all poisons act more rapidly and in smaller doses, if introduced subcutaneously or into the blood, than if taken into the stomach. But the substance obtains a practically hygienic importance only when the toxic action ascertained is also manifested on its introduction into the stomach.

Concerning many substances, *e.g.*, the seeds of weeds, the transformation products of schizomycetes, &c., we have in the first place to be content with deciding the special question if they are injurious to health, without being each time able to isolate the specially poisonous substances present. Also the exact toxico-physiological analysis of the process of poisoning with all modern appliances is not included in the scope of this book.

In examining the harmful character of gases, it is above all necessary that the poisoning should not be complicated by the deficiency of oxygen and the accumulation of carbon dioxide in the experimental room, and that the proportion of the gas in the air should be known. Preliminary experi-

ments on the action of large doses may be carried out in large glass cases of known volume, into which a measured volume of gas is driven. For exact experiments we require a glass chamber traversed by a current of air containing a constant and regulable proportion of the gas in question—requirements which are not always easily fulfilled. Persons interested in the question may be referred to my memoirs on ammonia and hydrochloric acid gas (*Archiv. f. Hygiene*, v.), and on chlorine and bromine (*Archiv. f. Hygiene*, vii.), where suitable apparatus are described. The inquirer may study gas-poisoning in his own person by developing the gases in question in a small chamber, mixing the air very well by means of a fan. For the determination of the proportions see "Air."

If an animal under experiment becomes ill, its urine and dung have to be tested for the poisonous substance, and the animal is observed with peculiar care, account being taken of the quantity of food consumed, &c. If it eats too little, so that the weight of the body (which must be frequently determined) is found to decrease, the attempt must be made to improve its appetite by a change of diet. What symptoms must be more especially attended to cannot be laid down universally and in a few sentences. It is very important to observe the animal frequently outside the cage. It is advisable not to depend too exclusively upon experiments on rabbits, since these creatures are little susceptible to many poisons, whilst their low degree of intelligence much interferes with the observation of delicate cerebral and nervous disturbances. I strongly recommend the use of cats, which are easily fed upon meat and milk. Immediately after death a dissection and macroscopic and microscopic examination of the corpse must be conducted, strictly according to the rules of pathological anatomy, followed, if needful, by a chemical examination of the organs.

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PART II.

SPECIAL INVESTIGATIONS.

SECTION I.

THE AIR.

A. Examination of the Air.

§ 96. Hygiene is especially concerned with the following properties of the air, whether in the open or in dwellings:—

I. *Physical Relations.*
   1. Temperature.
   2. Pressure.
   3. Movements of the air.

II. *Chemical Composition.*
   1. Proportion of watery vapour (conditions of downfall).
   2. Proportion of carbonic acid and other gaseous admixtures.

III. *Quantity of Corpuscular Matters.*
   1. Inanimate dust.
   2. Micro-organisms.

I. *PHYSICAL EXAMINATION OF THE AIR.*

1. Examination of the Temperature.

A. THERMOMETERS.

§ 97. For the determination of the temperature we generally employ liquid thermometers, *i.e.*, small glass vessels filled with mercury or alcohol, to which is affixed a fine tube,

sealed at the top, and exhausted of air. If the mercury-vessel of a thermometer is exposed to an elevated temperature, the mercury is strongly expanded, and a part enters the tube, which, on account of its narrowness, is filled for a considerable distance. The increase of the volume of the mercury occasions, for each equal increase of temperature, an equal rise of the mercurial column.

All bodies expand indeed according to the formula:

$$V_t = V_0 (1 + a\, t)$$

V_t, volume at t^0.
V_0, volume at 0^0, a being the coefficient of expansion.

The coefficient of expansion of different substances varies exceedingly. For mercury $a = 0{\cdot}00018$; for glass the linear expansion $= 0{\cdot}000008$; and the cubic expansion which is here in question $= 0{\cdot}000024$. The expansion of the glass may therefore be neglected in comparison with that of mercury. For further figures see Table II.

A good normal thermometer must fulfil the following conditions; exceptions in certain cases will be mentioned below.

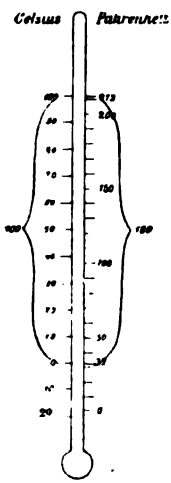

FIG. 41.—Thermometer-scales.

1. The *zero-* and the *boiling-points* must be indicated on the scale, and must agree with the true zero- and boiling-points.
2. The thermometer must be *sensitive*, *i.e.*, the mercury bulb must be small, and have thin sides, so that the mercury may be *quickly* heated.
3. The distance between 0 and the point of ebullition should be *great*, so that it is possible to read off small fractions ($\frac{1}{10}$) of a degree. If the mercurial reservoir (bulb) is small, a *narrow* capillary tube is necessary. The mass of the mercury in the tube should be vanishingly small compared with that in the bulb.
4. The tube should be everywhere of equal width, so that the degrees are of equal length.

The space from the freezing-point to the boiling-point of

water is for scientific purposes always divided into 100° parts, according to Celsius (centigrade); for domestic uses (temperature of rooms, baths, &c.), it is still [1] often graduated, according to Réaumur, in 80°. The mutual conversion of these degrees is effected according to the simple formula:—

$$t_R = \frac{t_C \cdot 4}{5} \text{ and } t_C = \frac{t_R \cdot 5}{4}$$

Where t_R and t_C stand respectively for the degrees of Réaumur or the centigrade as read or as sought for.

Example. 17° R. how many Centigrade? $\frac{17 \cdot 5}{4} = 21{\cdot}25$

$-15°$ C. how many Réaumur? $-\frac{15 \cdot 4}{5} = -12°$

Fahrenheit's scale is now used only in Britain and America, though so frequently that a knowledge of this scale is necessary.

Fahrenheit divides the space from the freezing-point to the boiling-point of water into 180°, therefore 9° Fahrenheit = 5° Centigrade = 4° Réaumur, but he does not begin to reckon from the freezing-point of water but thirty-two of his degrees lower, *i.e.*, at the lowest atmospheric temperature then observed, whence $\frac{32 \times 5}{9} = -17{\cdot}8°$ C. Hence his freezing-point is + 32°.

A calculation from Fahrenheit into C. and Réaumur is therefore effected as follows:—

$$\frac{(t_F - 32) \times 5}{9} = t_C \quad \frac{(t_F - 32) \times 4}{9} = t_R$$

Example. 100° F. how many Centigrade? $\frac{(100 - 32) \times 5}{9} = 37{\cdot}8$ Centigrade.

The inverse conversion is effected according to the formula:—

$$\frac{t_C \times 9}{5} + 32 = t_F, \text{ or respectively } \frac{t_R \times 9}{4} + 32 = t_F.$$

§ 98. In order to be satisfied of the accuracy of the position of the 0 and the ebullition-points in a thermometer we proceed as follows:—

1. 0° *Point.*—Since the 0° point is defined as the temperature of melting ice, the thermometer is plunged deeply among pieces of ice of the size of hazel-nuts or peas, which are

[1] In Germany.—*Translator.*

placed in a paper-filter supported in a large glass funnel. In this manner the bulb is constantly surrounded with water which has been flowing for some distance through ice. If the ice has been obtained in very cold weather (when it may have been cooled far below 0°) it is kept for some time in the room. From time to time the position of the mercury is examined without drawing the thermometer far out. In about a quarter of an hour the minimum-point is reached and is noted. We will assume we have found that the true 0° lies at + 0·2 of our thermometer scale.

The 0° point is gradually displaced upwards in new thermometers by a very slow contraction of the bulb owing to the pressure of the air.

After every prolonged exposure to a very high temperature the glass bulb (the volume of which is also affected by the heat, though very slightly) remains for a time rather larger, and the 0° point lies temporarily rather too low ("depressed 0° point").

2. *Boiling-point.*—The thermometer is placed in a copious current of steam enclosed within narrow boundaries, as the temperature of steam depends merely on the barometric

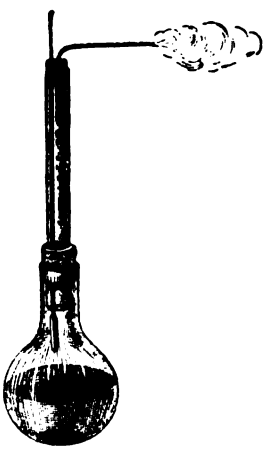

FIG. 42.

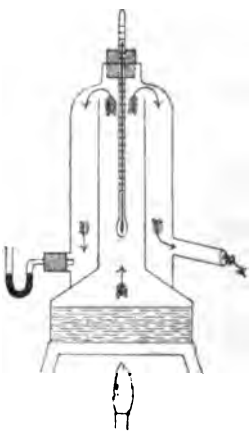

FIG. 43.

pressure, whilst the temperature of boiling water is in addition affected by the form, the superficies, and the material of the containing vessel. Two arrangements have been introduced. The one apparatus (Fig. 42) consists of glass,

with caoutchouc stoppers, and can be improvised at any moment. The second apparatus (Fig. 43) is made of sheet-brass, and on account of its opacity its structure is less distinct in reality than it appears in the diagram (Fig. 43). In both these apparatus the thermometer is immersed in the steam almost for its entire length, only the part from 90° projecting out for examination. The track of the steam is indicated by arrows; in the brass apparatus the column of steam surrounding the thermometer is still further enclosed in a stratum of steam, and thus protected from refrigeration. After the water has begun to boil, from ten to fifteen minutes must elapse before the maximum-point is reached. The brass apparatus yields by far the more accurate results.

The barometer is then observed, and on reference to Table I. it is seen at what temperature water boils at the pressure shown. Or we calculate the theoretical boiling-point according to the formula $t = 100 - 0{\cdot}0375\,(760 - b)$, where b denotes the height of the barometer read off and reduced to 0°.

If, *e.g.*, our thermometer after fifteen minutes stood at 98·7°, with a reduced pressure of 750 *mm.*, and if the table shows that at 750 *mm.* water boils at 99·6°, it follows that 99·6° must stand in place of 98·7°, and further that in place of 100° there ought to stand 100·9°, *i.e.*, the thermometer in the neighbourhood of the boiling-point indicates 0·9° too low.

§ 99. The examination of the uniform calibre of the capillary tube endangers the instrument if attempted by an inexperienced operator; but as, in addition to a control of the boiling- and the freezing-points, a control must also be effected at least at a few intervening points of the scale, it is preferable to compare the thermometer with a standard instrument (borrowed, if necessary), the capillary tube of which has been carefully calibrated, and which is accompanied by a table of corrections. For thermometers without a 0° point (so-called fever thermometers, &c.) this is the only check possible.

For this purpose the standard thermometer and the instrument to be tested are fixed in two supports, and their bulbs are allowed to plunge, side by side, deep into a wooden

pail of water; or before immersion we secure together both thermometers by means of a caoutchouc ring, which is cut from a wide tube. After some minutes (ten at most) the position of the thermometers will have become constant. They are read off, and some hot water is added, which is thoroughly mixed with the entire volume of water by blowing in air through a wide glass tube. Additions of hot water are repeated several times.

Example.—Let the following values be found for the thermometer to be gauged :—

Normal Thermometer (Or corrected indications of the normal thermometer).	*Thermometer in Question.*	*Difference.*
0	+ 0·2	+ 0·2
10·3	10·4	+ 0·1
15·6	15·6	+ 0·0
20·7	20·5	− 0·2
34·0	33·6	− 0·4

From these observations we calculate a table of corrections for the thermometer under examination. We assume that the difference observed at the point tested prevails from the middle of the foregoing to the middle of the following interval of temperature.

The middle of the first interval 0·2 to 10·4 is 5·3, that of the second 13·0, of the third 18·1, of the fourth 27·1. Hence the table runs :—

Instead of the temperature as read off we must take :—

In the interval.	0·2 − 5·3	t + 0·2
	5·4 − 13·0	t + 0·1
	13·1 − 18·1	t + 0·0
	18·2 − 27·1	t − 0·2
	27·2 − 33·6	t − 0·4

If no other observations are available (one such should in this case decidedly be interpolated between 20·5 and 33·6), the first correction must be adopted for the values below 0°, and the last for at least some extent above 33·6°.

§ 100. For very low temperatures (below −30°) alcohol thermometers must be used, as mercury congeals at −39·4°, and for some extent above this point its contractions are irregular. Alcohol does not loose its fluidity until −166°. At mean and higher temperatures alcohol thermometers are

inaccurate, as with a rising temperature alcohol expands more and more strongly, and boils at 68·3°. A little alcohol readily distils into the point of a thermometer (especially if horizontal); if the drop does not flow back when placed for some time in a perpendicular position, the instrument must be standardised afresh. Mercury evaporates at 360°, and above 300° a mercurial thermometer is inaccurate. If higher temperatures have to be observed, which is rarely needed for hygienic purposes, air thermometers (the expansion of a volume of air shut in by means of mercury) or pyrometers come into use. A favourite kind of pyrometer (Prinsep) consists of a collection of alloys of platinum having different melting-points. Each test lies in an infusible crucible; its fusion indicates the temperature concerned. (See Belz, *Die Pyrometer*, Berlin, 1891.)

Metallic thermometers consist of slips of sheet brass and steel rolled upon each other in a spiral form in such a manner that the brass lies on the inner side of the coils and steel on the outer side. The centre of the spiral is fixed; the other end communicates its movement to an index which plays along a scale graduated with the aid of a mercurial or air thermometer. As brass expands in heat much more strongly than steel, the spiral uncoils itself a little at higher temperatures, and is drawn together at a lower heat. If the spiral consists of many folds, and if its mass is small (thin slips of sheet metals), the resulting thermometers are very sensitive, and their indications are given with extreme rapidity.

For the measurement of the temperature of the body, metallic thermometers of the form of a small watch have been lately recommended (Immisch), and are said to give satisfaction.

It is often interesting to ascertain the highest and the lowest temperature during a certain time. For this purpose we use:—

§ 101. **Rutherford's Maximum and Minimum Thermometer.**—Two thermometers are fixed horizontally upon a

glass plate, the one above the other; the bulb of the one pointing to the left, and that of the other to the right. The one is a mercurial thermometer, the column of which propels before it a minute steel rod, and leaves it lying at the furthest point, which it reaches (maximum point). The other contains alcohol, and has as index a small rod of glass slightly extended at each end. When the temperature rises the alcohol easily flows past this rod, but carries it along on returning. so that the rod is left at the lowest position of the thermometer (minimum temperature). In order to make the instrument ready for use, the glass plate is turned so that the mercurial bulb looks downwards as in the ordinary suspension of a thermometer, whilst the alcohol thermometer is almost reversed with the bulb upwards; the indices then adjust themselves by the action of gravitation. Latterly it is found preferable to fix the two thermometers each upon a separate slip of glass.

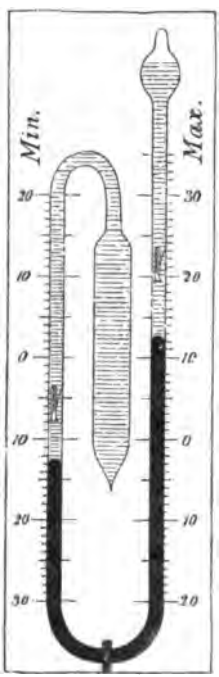

FIG. 44.—Maximum-minimum Thermometer.

§ 102. At present the maximum and minimum thermometer of Six (Thermometograph, Fig. 44) is very often used. It is an alcohol thermometer with an ascending U-tube, in which the alcohol propels as an index a long thread of mercury. At both ends of the mercurial thread there are small glass rods with an iron nucleus, fixing themselves against the sides of the glass by means of elastic, bristle-like projections. These rods mark the two extreme positions of the mercurial thread. In order to effect a prompt return of the mercurial thread on the cooling of the alcohol, and to ensure a complete contact of the mercury and the alcohol, one end of the ascending tube is slightly expanded, and contains a

small quantity of alcohol and alcoholic vapour. The tension of the vapour acts like a slight spring upon the regularity of the movements of the mercury. The thermometer has a twofold scale. If the alcohol thread, and consequently the left end of the mercurial thread, stands at 0°, the right end of the latter must also be marked with 0°. The mark + 10 must lie to the left in the descending limb, exactly so far below the point 0 as it stands above it on the right in the ascending limb. Whilst, therefore, the two ends of the mercurial thread stand always equally at equal points of the scale, the glass rod to the left indicates the minimum, and that to the right the maximum, temperature. At the commencement of each series of observations the rods are brought to the ends of the index thread by means of a horse-shoe magnet.

In mercurial thermometers for measuring the temperature of the body, a globule of air is often enclosed in the mercurial thread, as recommended by Ehrle. The anterior separate part of the mercurial thread only rises when this air is compressed until it becomes imperceptible. As the mercury falls at a lower temperature, this bubble of air expands; the superincumbent part of the thread remains as an indicator of the highest temperature which has been reached, whilst its lower half retires. Each time before the thermometer is used it must be seized by the top, and short strokes must be made with it in the air, to bring the separated thread of mercury into such a position that the upper end of the portion of the thread which acts as an index lies lower than the maximum temperature probable. Lest the air-bubble should penetrate into the mercurial bulb, the ascending tube is curved repeatedly in the part above the bulb.

The Bavarian meteorological stations make use of mercurial maximum thermometers suspended in a slightly inclining position, in which a glass point projects into the mercury at a part of the tube which is slightly expanded. On the expansion of the mercury the valve-like action of this point is readily overcome. On cooling it produces a rupture of the mercurial thread, so that the front end of the thread serves as an index, and has to be brought back by an upright position, or by shaking and tapping.

Very recently Zeiss, of Jena, has produced a normal glass, distinguished by a thread of violet glass melted into it. It shows no perceptible after-effects of protracted exposure to heat and cold, and serves for the production of unchangeable bulbs for thermometers. His "blood-thermometers," made from this material, admit of a control of the 0° point by the simple expedient of slightly expanding the lower part of the ascending tube.

Metal thermometers may also serve very well as maximum and minimum thermometers. Against the finger which is displaced by the movement of the metal spirals there are placed to the left and the right two light metal indices, which turn on a central action with moderate friction. If at the commencement of the experiment the two indices are placed with the hand in contact with the finger left and right, we can infer the highest and the lowest temperature from their position after an interval of time, since the one index gives the extreme position to the right and the other the extreme left-hand position of the finger.

B. THE OBSERVATION OF THERMOMETERS.

§ 103. In general the determination of the temperature of the air is required with the exclusion of the action of the sun's radiant heat upon the thermometer.[1] Thermometers are therefore always to be placed in the shade. For regular observations it is preferable to associate an ordinary thermometer, a maximum and minimum thermometer, with a psychrometer and a hair hygrometer in a sheet-metal case placed in front of a north window, protected against the sun, if needful, by especial screens, but freely open to currents of air. (See instructions for the Bavarian meteorological stations, where good illustrations may be found.) The case protects the instruments from rain, &c. It is fixed 3 *m.* from the ground and 0·5 *m.* from the window. For the convenience of observations a simple mechanism allows it to be brought close to the closed window, and afterwards to be returned to its place. For readings by night and in bad weather this arrangement is almost indispensable. For meteorological purposes the temperature is generally read off three times, 8 A.M., 2 and 8 P.M. At 8 P.M. the minimum of the previous night, and the maximum of the day just ended, are also read. A day therefore extends from midnight to midnight, but the

[1] Upon mercurial thermometers made entirely of glass and suspended freely, the sun's rays have scarcely any action on account of the good reflection of the mercury. If the thermometer is fixed against any material which absorbs heat it rises at once.

minimum and maximum thermometer is adjusted at 8 P.M., consequently four hours before the beginning of the day. The daily average is obtained according to the method which has been hitherto in universal use by adding the temperatures at 8 A.M., 2 and 8 P.M., and the minimum (which approximately represents 2 A.M.), and dividing the sum by four. Values with a — sign are of course subtracted instead of added.[1]

Examples :—

I. *Old Method.*	II. *Old Method.*	III. *New Method.*
8 A.M. 12·0°	− 6·0°	8 A.M. −4°
2 P.M. 20·2°	+ 3·5°	2 P.M. +8°
8 P.M. 14·4°	+ 0·1°	10 P.M. +1°
Minimum 8·4°	− 8·0°	10 P.M. +1°
55·0 : 4	3·6 − 14·0 = − 10·4 : 4	10 − 4 = +6 : 4
Mean 13·7°	Mean − 2·6°	Mean +1·5°

For accurate observations of the temperature of enclosed spaces (rooms, &c.), it is necessary to exclude the radiation of stoves, lamps, &c., by the interposition of screens, and then to measure the temperature at various places near the ceiling and on the floor, at the window and near the stove, and to take their mean, dividing the total value by the number of observations.[2]

§ 104. Hygiene has hitherto paid little attention to the determination of radiant heat. Pouillet's pyrheliometer determines the heat irradiated by the sun upon a given surface in a given time by fixing a silver box blackened on the outside and filled with water to a heliostat, which always keeps it in a position normal to the direction of the sun's rays,[3]

[1] It is recently proposed to observe and add 8 A.M., 2 and 10 P.M., and again 10 P.M., dividing the sum by four. (Bezold and Erk.)

[2] Self-registering thermometers have been devised of the most varied constructions depending in part on the use of photography, but they are not so available on account of their high price as to render a minute description necessary. The movement of the finger of a metal thermometer may be easily caused to register the temperatures upon a revolving drum.

[3] The angles of incidence of the sun's rays, the temperatures being equal, appear to have no inconsiderable effect on the well-being of plants. Animals vary their positions so often and so greatly that the effects in their case are less easily determined. (See Veitch "Manual of Orchidaceous Plants Cultivated under Glass.")—*Translator.*

and then allows the increase of the temperature of the water to be determined. The number of the calories irradiated can thus be determined. An expensive apparatus.

Fig. 45. Vacuum Thermometer.

For hygienic purposes we generally use a mercurial maximum thermometer, the bulb of which is either made of black glass or coated with lamp-black, and sealed up in a wider glass tube, the air of which has been exhausted. The black coating renders the bulb capable of an energetic absorption of radiant heat, whilst the surrounding vacuum excludes as far as possible any loss or gain of heat by conduction.

The thermometer rises until its bulb loses in a unit of time as much heat to the glass casing as it takes up from without. The greater the intensity of the radiant heat so much higher does the vacuum thermometer rise than an ordinary thermometer, this difference being therefore a measure for the radiation of heat.

In order to expose the bulb of the vacuum thermometer to the sun's rays in the most favourable manner, it should be fixed in a horizontal position.[1]

2. Investigation of Atmospheric Pressure.

A. BAROMETERS.

§ 105. As it is well known, Toricelli found that if a tube of any length (about 1 *m.*), closed at one end, is filled with mercury, closed with the finger, placed in a trough of mercury and the finger withdrawn, there always remains in the tube a column of mercury of about 760 *mm.* in height, above which there is a vacuum. This is the most primitive barometer. The mercurial column is held in equilibrium by the pressure of a

[1] Most important data on the atmospheric temperature are obtainable by means of the radiograph of Mr. D. Winstanley, which registers at once both the intensity and the duration of sunshine. For a full account of this instru-

column of air of the height of the atmosphere. If this pressure increases, more mercury is raised into the tube, the barometer rises, and inversely.

If we were to place a measuring-rod alongside of the column of mercury, so as to measure its length, we should obtain tolerably accurate results; for the level of the mercury in the trough is scarcely changed, on account of its

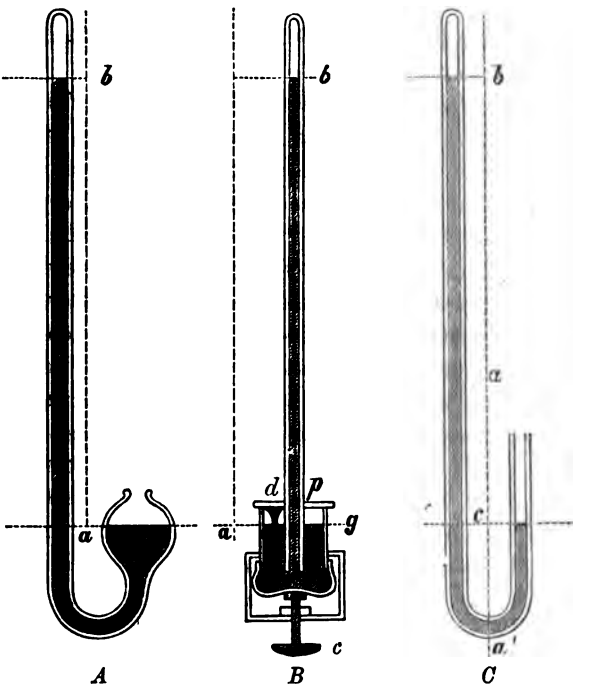

FIG. 46.—Barometer.

large surface, when the level in the tube varies. The 0° point is therefore almost constant. But as such an apparatus is inconvenient, there is often used in its place a smaller vessel, of course open. These instruments (cistern-

ment, with illustrations, we must refer to the *Journal of Science*, third series, vol. iii. 1881, p. 218.

This instrument is not to be confounded with the radiometer of Mr. W. Crookes, which has a different purpose. Nor should the *Journal of Science* quoted be mistaken for the *American Journal of Science*.—*Translator.*

barometers) are very inaccurate if used without further correction, as in common dwelling-house barometers (Fig. 46, *A*). Here every fluctuation of the height of the mercury in the tube alters the zero-point of the scale. Thus if, *e.g.*, the height of the mercury in the tube sinks, the level in the vessel rises, and the fall in the tube is to a small extent compensated.

§ 106. For scientific purposes we use two forms of the cistern-barometer.

1. *Fortin's Cistern-Barometer* (Fig. 46, *B*). The cistern into which the tube dips has a bottom of leather; by turning the screw *c*, the level of the mercury can be so far raised and lowered that it always exactly touches the ivory point *d*, the level being therefore kept constant. A scale, the zero-point of which corresponds to the ivory point, is etched upon the tube. At *p* the tube is inserted into the cistern so as to be mercury-tight but not air-tight. For safe conveyance, even on long journeys, the leather is screwed up so far that the cistern and the tube are both filled with mercury. The whole apparatus is enclosed in a solid case of brass.

2. *Kappeller's Station-Barometer* has a fixed cistern with a variable level, like the ordinary domestic barometer. But as the proportion of the section of the tube and of the cistern is known, and the volume of mercury is constant, there results a simple correction for calculating the true height of the barometer.

If B is the mean height of the barometer according to the altitude of the place,
b the height of the barometer as read,
b_c the true height of the barometer sought,
d the diameter of the tube,
D the diameter of the cistern,

$$b_c = b - \frac{d^2}{D_2 - d^2}(B-b);\ \frac{d^2}{D-d}$$

is a constant $= a$ for each instrument, so that the formula now runs: $b_c = b - a(B-b)$. The value a is given on the barometer, or it is carried out equal to the graduation of the scale with reference to the factor a, *i.e.*, the degree-marks are at the distance of rather less than 1 *mm*.

One fault is common to all barometers, independent of any difference of level in the cistern. If we use mercury and glass in narrow tubes, there occurs a considerable capillary depression. If the width of the tube is 4 *mm.*, it still amounts to 1·6 *mm.* of mercury in height. At 20 *mm.* it is only 0·025 *mm.* In order to eliminate capillary depression we must use either a very wide tube, or at least one which is enlarged at its upper part, as is now the case even in the superior kinds of domestic barometers.

§ 107. For the complete removal of every influence of the vessel and of capillary depression we use the syphon barometer (Fig. 46, *C*). Here the mercurial column in the long limb counterbalances the atmospheric pressure *plus* that of the column in the short limb, consequently the atmospheric pressure is measured by the difference of the lengths of both columns. The depressions in both limbs are equal, and consequently annul each other.

If we read off on the long limb 780 *mm.*, and on the short limb 30 the aerial pressure = 750. We may here also notice that the sum of the two readings 780 + 30 = 810 is constant for each barometer, so that we have thus always a check on the possible correctness of our readings.

In the simpler syphon barometers a scale is attached, or is preferably engraved on the glass, and we may thus read how high the mercury in each limb stands above the zero-point of the scale, and calculate the difference of both readings. In the more expensive instruments the scale or the barometer tube itself is movable, so that before each reading the convexity of the mercury in the short limb can be adjusted to the zero of the scale. On reading off the height in the long limb we have the true height of the barometer without any calculation.

To prevent the scale becoming opaque and blackish by the oxidation of the mercury in the short limb, the syphon barometer, when not in use, is suspended in an inclined position, and only brought temporarily to a perpendicular for reading off. For removal the barometer is inclined so far that the vacuum is entirely filled with mercury.

§ 108. In reading off a barometer we always take the position of the summit of the convexity of the mercury. To facilitate this in superior instruments we move a horizontal mark (a wire or metal ring) until it exactly coincides with the summit of the mercurial convexity and read off with what degree of the scale the mark coincides. For placing a barometer we suspend it before a brightly lighted wall, but we do not throw a bright light upon the instrument itself. The temperature at the place where the instrument is suspended should be moderate and uniform. It may be mentioned that the surface of the mercury in the barometer-tube always displays a convexity, due to the capillary repulsion of the glass, and which by no means indicates a tendency of the mercury to rise. It is evident that a barometer can give accurate indications only if it hangs exactly perpendicularly.

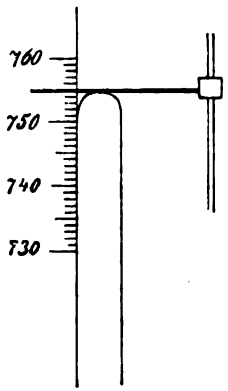

FIG. 47.—Reading a Barometer.

In order to make the indications of one barometer comparable with those of another, two further observations or calculations have to be made.

1. *Observation of Temperature.* — As mercury expands strongly on a rise of temperature, its specific gravity is considerably modified, and a higher column of the warm and specifically lighter mercury is raised accordingly.

All observations must therefore be reduced to the density of mercury at 0°, and we always read off t, the temperature of the place where the barometer is suspended, before reading the barometer, in order not to allow the thermometer to rise whilst the barometer is being read off.

If the length of the barometric column at $0° = h_0$, then at t_0 it would be $= h_0 + h_0 \times t \,.\, 0{\cdot}0001815 = h_1$ or $h_0\,(1 + t \,.\, 0{\cdot}0001815) = h_1$; or

$$h_0 = \frac{h_1}{1 + t \,.\, 0{\cdot}0001815}$$

where 0·0001815 is the coefficient of expansion of mercury. The calculation may be greatly simplified by means of Table IV.

Example: At 10° the height of the barometer read off is 751 *mm.*; what will it be at 0°?

$$h_0 = \frac{751}{1 + 0{\cdot}001815} = 749{\cdot}8$$

If the barometer scale is engraved upon metal its elongation compensates to a small extent for the error occasioned by the expansion of the mercury. For hygienic purposes the elongation of the scale may be disregarded.

2. *Reduction to the Level of the Sea.*—The higher we ascend above the sea-level the more the height of the barometric column decreases with the height of the atmospheric column. If it is therefore required to form an opinion whether the atmospheric pressure is relatively greater at one of two places situate at different altitudes than at the other, we reduce the pressures observed at both places to the sea-level. These reductions are indispensable for the meteorologist in questions of a prognosis of the weather.

As in the lower strata of the atmosphere a column of air of about 11 *m.* in height holds in equilibrium a pressure of 1 *mm.* mercury, the correct reading of the barometer will be approximately h_n:—

$$h_n = h + \frac{x}{11}$$

where h is the degree of the barometer as read off, and x the altitude of the place above the sea-level in metres.

The factor, which is here assumed = 11, varies of course with the aërial pressure and the temperature. Table V. gives the more accurate values of different conditions of pressure and temperature.

At Munich, 528 *m.* above the sea-level, the degree of the barometer observed at 20° and reduced to 0° was 710, what will it be at the sea-level?

$$h_n = 710 + \frac{528}{12{\cdot}16}$$
$$h_n = 710 + 43{\cdot}4$$
$$h = 753{\cdot}4.$$

As a matter of course, whilst the reduction of the barometer to 0° must always be effected, that to the sea-level need only take place in peculiar cases, especially when utilising meteorological observations from extensive regions, but not in the reduction of the volumes of gas, &c.

§ 109. In addition to the mercurial barometers, which, if carefully used, are very lasting, extensive use has been latterly made of the so-called aneroid barometers, which depend on the fact that an elastic metallic case exhausted of air is compressed the more strongly the greater is the atmospheric pressure. Two modifications of this instrument are in use, and are here described in general terms.

1. *Bourdon's Baromètre Metallique* (Fig. 48).—A metal tube exhausted of air (shaded in the figure) is bent round almost to a circle, and is fixed at the middle. The pressure of the air on its larger external circumference predominates in opposition to that upon the smaller internal circumference. If the pressure rises the ends of the tube approach each other, and their movement, communicated to a lever, increases the tension of a watch-spring; on it is placed an arc of a toothed wheel, which catches into a toothed wheel carrying a finger. The scale upon which the finger plays is graduated empirically according to a mercurial barometer. A cheap, convenient, portable, but generally not very substantial apparatus.

FIG. 48.—Bourdon's Baromètre Metallique.

2. *Goldschmidt's Aneroid Barometer.*—An improvement on Naudet's (Fig. 49). A strong metal box, *a*, exhausted of air, supports a thin lid, soldered on, and rendered concave by the pressure of the air. A rod, *b*, is soldered on, and has on its horizontal arm a small block, *c*, with a mark. The spring, *e*, presses a second small block, with a mark, *d*, in such a manner that at a certain position of the box-lid

the two marks coincide, *e.g.*, at the pressure of 760 *mm.* If the pressure increases, the lid is forced in more strongly, and the mark, *c*, is depressed. The micrometer screw, *f*, with a graduated margin, *g*, is now turned until the marks again coincide, and then read off to find how many degrees the graduated edge of the screw has turned past a fixed index, *h*. We then find in a table to what increase of atmospheric pressure this corresponds. The influence of the temperature, by the rise of which the lid of the box is rendered more sensitive to pressure, is eliminated by not absolutely exhausting the box. The increasing tension of the residual air counteracts the decrease of elasticity of the metallic membrane. A lasting, but inexpensive instrument, very useful if frequently compared with mercurial barometers.

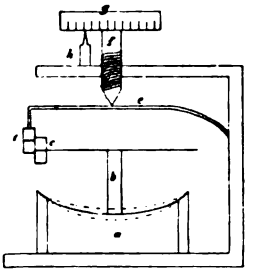

FIG. 49.—Goldschmidt's Aneroid.

Graphic representations of barometric levels may be produced without much difficulty, but have little hygienic importance (see Günther, *Meteorologie*, 1889, p. 47, &c.).

B. MANOMETERS.

§ 110. If it is required to observe the pressure which gases or liquids in closed vessels, tubes, &c., exert upon their surroundings, we use manometers. As such there are generally employed U-tubes, open at both ends, which for the determination of strong pressures ($\frac{1}{10}$ to at most $\frac{1}{2}$ atmosphere, steam, water, compressed air) are filled with mercury; for low pressures (up to about $\frac{1}{10}$ atmosphere) with coloured water, oil, petroleum, &c. The pressure, positive or negative, is measured by the distance of the convexities in the central

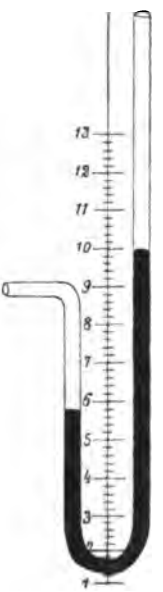

FIG. 50.—Manometer for Low Pressure.

and the external limb, and are expressed in millimetres of mercury or water. 760 *mm.* of mercury pressure = 1 atmosphere of over-pressure. As mercury is 13·596 times heavier than water, mercurial pressure is converted into water pressure by multiplication with 13·596: on the other hand, water pressure divided by 13·596 = mercurial pressure. In Fig. 50 we read off 99·5 − 58 *mm.* = 41·5 *mm.* mercury = 41·5 . 13·596 = 564·2 *mm.* water pressure = $\frac{41·5}{760}$ = 0·0546 atmosphere.

The observations on a manometer filled with a liquid lighter than water (oil, &c.) are recalculated into water pressure by multiplying the millimetres read off by the specific gravity of the oil.

We can also use manometers of metal (*e.g.*, in steam boilers), curvatures of metallic membranes being increased or reduced by an increased or a diminished pressure. But in hygiene we may generally be satisfied with the inexpensive one obtained by bending a glass tube, which merely requires filling and fixing to a piece of wood, along with a millimetre scale on paper.

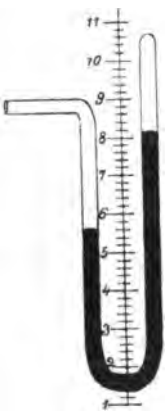

FIG. 51.—Air-manometer.

If a strong pressure, *e.g.*, that of water in many waterworks, has to be measured, we seal up one limb (the peripheric limb) of a mercurial manometer (Fig. 51), and use the compression of the air enclosed in this limb as a scale for measuring the pressure. At 1 atmosphere over-pressure the volume of the air decreases to ½, at 2 atmospheres to ⅓, at 6 to ⅐, &c. Only stout glass tubes can serve for such experiments. In Fig. 51 the pressure amounts to 1½ atmospheres.

Determination of Minimum Differences of Pressure.

§ 111. Where we no longer obtain any indications with the ordinary manometer, Recknagel's Differential Manometer still affords very distinct indications of differences of pressure. It depends on the idea firstly to elongate the liquid taken up by causing it to ascend not vertically but in a slanting position, and selecting the central limb so wide and the peripheric limb so narrow that the level of the former is not perceptibly affected even by the strongest indications in the peripheral limb. From Fig. 52 it will be seen how the indication is magnified by the sloping position of the ascending tube. At an incline of 3 to 5 per cent., 1 *mm.* difference of pressure represents an indication of 19·1 *mm.* to 11·4 *mm.*

The filling of the brass box which acts as a central limb, and which is about 10 *cm.* in diameter, is best effected with petroleum. The thinner limb is placed more or less aslant, according as a greater or smaller sensitiveness is intended. In order to gauge the scale of the inclined glass tube, which is graduated in centimetres and millimetres for any given inclination, the box is filled with petroleum so far that it becomes visible in the sloping tube, and its position there is read off. A glass flask full of petroleum, along with a funnel, are then weighed off together; so much petroleum is then poured in that a strong indi-

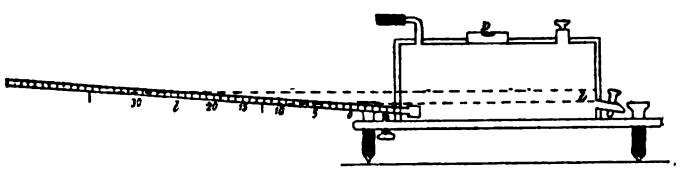

FIG. 52.—Recknagel's Differential Manometer.

cation becomes visible in the tube and the flask; the remaining petroleum and the funnel are then weighed back.

If p grammes of the vol. v are added,
g the section of the box in square centimetres,
n the indication in the tube of the manometer in centimetres,
d the specific gravity of the petroleum,
the indication of n centimetres as read off corresponds to an increase in the column of petroleum in the box of $h = \frac{v}{g} = \frac{p}{d.g}$ *cm.*

In order to convert petroleum pressure into water pressure, we calculate what water-column (h_1) corresponds to a petroleum column of h height (the heights are inversely as the specific gravity).

$$h_1 : h = d : 1$$

$$\text{therefore} \quad h_1 = dh \text{ or } h_1 = \frac{d \cdot p}{d \cdot g}$$

or n centimetres indication on the scale represents a water pressure of $\frac{p}{g}$ *cm.*,

or 1 *mm.* indication $= \frac{p}{g \cdot n}$ *mm.* water pressure.

As the specific gravity of petroleum decreases at medium temperatures by 0·0007 for every degree of heat, and rises inversely, the following correction for temperature must be applied. If the apparatus has been gauged at 12° with petroleum of specific gravity 0·8070, and if we afterwards observe at 15°, we must multiply the result by $\frac{8070-(3.7)}{8070}$, *i.e.*, $\frac{8049}{8970}$; if we observe at 9°, we multiply by $\frac{8070+(3.7)}{8070}$, *i.e.*, $\frac{8091}{8070}$.

For a simple gauging of the apparatus with an especial auxiliary instrument, see *Archiv f. Hygiene*, xi., in Schönwerth's work. A modified differential manometer filled with alcohol is there also recommended.

The apparatus does excellent service if, *e.g.*, the rarefaction of air in a chimney, the action of ventilation channels, the pressure of the wind on walls, the inrush of the ground-air through a cellar floor, &c., have to be measured or merely manifested. The use of the instrument is exceedingly simple. By means of the metal arm an iron pipe provided with numerous lateral holes is firmly connected with a stout, short, new caoutchouc tube, and driven into the wall, &c., in which investigations as to the pressure of the air have to be conducted. The connection with channels, &c., is of course more simple. Here it is sufficient to insert a glass tube, air-tight, into the channel, and connect its other end with the manometer. In setting up the apparatus, the differential manometer must be placed exactly horizontal by means of a level which accompanies the apparatus.

The application of the apparatus to measure small velocities of wind will be considered in § 115.

3. Movement of the Air.

§ 112. In the open air we are from various reasons interested in—

1. The strength.
2. The direction.
3. The duration of aërial currents.

In dwelling-rooms we have frequently to determine where

the air enters or takes its exit, as also to ascertain the quantities of air concerned.

The direction of the wind often differs in the higher and the lower strata of the air. In the former we may judge by the movement of the clouds; in the lower strata by weathercocks, sometimes also the direction of smoke from the chimney, or of flags and streamers.

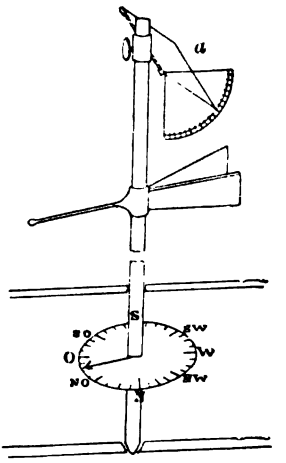

FIG. 53.—Weathercock.

Weathercocks for scientific purposes are generally fitted with an axle prolonged through the roof, and turning within in a journal. An index on the axle allows us to see, inside the house, the position of the vane, without any inconvenience from bad weather or darkness, and indeed much more accurately than it is possible by a direct observation from below.

The wind is named according to the direction whence it comes, streams of water inversely by the directions whither they flow.

In the compass-card (Fig. 54) the quadrant from W. to S. is, *e.g.*, read as follows: west, west-south-west, south-west, south-south-west, south. East is denoted by E., according to international agreement. For hygienic purposes a statement of the eight principal directions is sufficient. An approximate determination of the force of the wind in the open is often effected without apparatus by observing its effects upon trees. Of Beaufort's nautical scale of twelve degrees we use on land only six numbers, which correspond approximately to the accompanying velocities of the wind:—

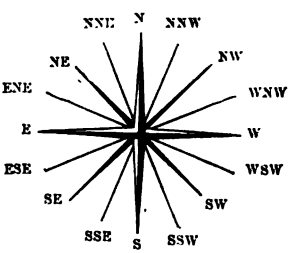

FIG. 54.—Compass-card.

Degree.	In Words.	Effects.	Speed in Metres per Second.
0	Calm.	Smoke rises perpendicularly; no leaf moves.	0–0·5
2	Slight.	Can be felt; moves streamers and light leaves.	0·5–4·0
4	Moderate.	Extends a streamer; moves leaves and small twigs of trees.	4–7
6	Fresh.	Moves larger twigs of trees.	7–11
8	Strong.	Moves large branches and weak stems; opposes walking in the open.	11–17
10	Storm.	Shakes entire trees; breaks branches and moderate trunks; uproots small trees.	17–28
12	Hurricane.	Unroofs houses; breaks down well-built chimneys; breaks and uproots large trees.	28–40

It would be very desirable if the actual velocity of the wind in metres could be substituted for the empirical scale, *e.g.*, NW_4 = "moderate north-west wind."

More exact determinations of the strength of the wind are hitherto attempted only at meteorological stations. For this purpose there is used at the angle of deflection a plate of sheet-metal (Fig. 53, *a*), fixed across the direction of the wind, hanging perpendicularly downward when at rest. Each such apparatus must be graduated empirically. The metal plate is kept at right angles to the direction of the wind by being secured to a weathercock.

§ 113. On account of the frequent variations of the strength and direction of the aërial currents, the observations just mentioned can often scarcely be instituted. Every few minutes the weathercock and the anemometer often show a different position. Numerous, and, if needful, graphic observations have to be made, and frequent mean values have to be calculated.

By means of Robinson's anemometer it is possible, quite independent of the most frequent alterations in the direction of the wind, to determine its mean velocity during any given

time. The wind may come from whatever side it happens, it turns the cups in the same direction with a velocity which depends only on its strength. This results because the hollows of the cups always afford the wind a much better surface to act upon than the convex sides. In whatever direction the wind may come, it always strikes with equal strength a concavity and a convexity of two cups fixed on one arm; it turns, therefore, always in such a direction that the convex sides move foremost. By a complicated counting mechanism not easy to explain in detail, the number of

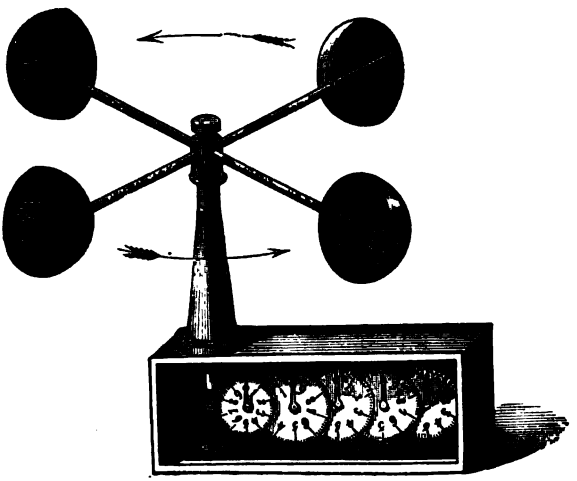

FIG. 55.—Robinson's Anemometer.

rotations in a given time is determined, and from the hourly number of rotations the mean velocity of the wind per second is calculated in metres, according to a formula which accompanies each instrument.

As a matter of course such apparatus must be placed so as to be fully open to the wind, protected by suitable arrangements against rust and against being clogged up with dust, and must be so substantial as to defy any storm. If these conditions are complied with, the indications form a valuable complement to the other ordinary observations of the wind. Self-registering movements are rarely wanting in good apparatus.

§ 114. In order to make observations on currents of air in rooms, we have generally to examine very feeble atmospheric movements, whence more sensitive appliances are necessary. A good indication is given by the smoke of a cigar blown gently out of the mouth (the smoke of a burning cigar always ascends, on account of its high temperature, if not deflected by powerful currents of air). Non-smokers may make use of the vapour of a benzoin fuse.[1]

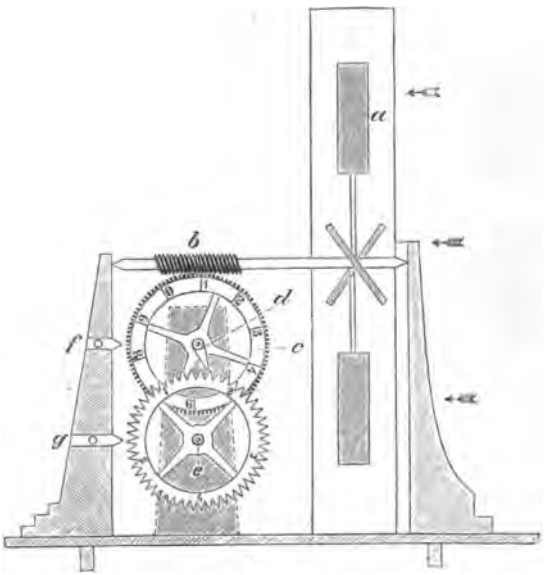

FIG. 56.—Combes' Anemometer.

The velocity of a current of air of a constant direction is determined by means of the anemometer of Combes. This small apparatus consists of a light vertical wheel with four spokes, with paddles of mica fixed obliquely (Fig. 56, *a*), in the axis of which is cut an endless screw, *b*. This catches into the teeth of the wheel *c*, which at every rotation of the paddle-wheel moves one tooth past the fixed index *f*. As often as the first wheel (which has 100 teeth) has made one

[1] Benzoin fuses are made by steeping soft cotton wicks in tincture of benzoin, and drying them. Such fuses, if lighted, burn steadily on, and give out a dense aromatic vapour. They are quenched by pressure.

complete rotation, it propels, by means of the tooth *d*, the second toothed wheel by one tooth further past the index *g*; such a degree shows, therefore, 100 rotations of the paddle-wheel. Before every experiment the counting works are cut off by a mechanism which varies in different apparatus (*e.g.*, by lifting the endless screw from the toothed wheel). The result is read off; the hundreds and thousands on the wheel *e*, and the tens and units on the wheel *c*. In our figure the number to be read is 0385. The anemometer is then held vertically, perpendicular to the aërial current to be examined, and so that it strikes the open side of the wheel, and not that partially covered by the counting-wheels. We wait the beginning of a minute with a watch which shows seconds, introduce the counting-works at this moment, dismount it after the lapse of a minute, read off the wheel-work, and subtract the two numbers read off before and after the experiment. It is very convenient if an assistant gives the beginning and end of the minute. If the number of rotations per minute has been found = *n*, we calculate *v*, the velocity of the wind in metres, according to the following formula:—

$$v = \alpha + \beta . \frac{n}{60}$$

Here α and β are two constants which have to be specially determined for each instrument. α is called the resistance of inertia; it represents that part of the power of the wind which is necessary to overcome inertia. If the velocity of the wind is not greater than α, the wheel does not turn, and n remains = 0. β represents the resistance of friction.

If in an apparatus the formula is, *e.g.*,

$$v = 0{\cdot}4 + 0{\cdot}143 . \frac{n}{60}$$

and if n = 450 rotations per minute,

$$v = 0{\cdot}4 + 0{\cdot}143 . \frac{450}{60}$$

$$v = 0{\cdot}4 + 0{\cdot}143 . 7{\cdot}5$$

$$v = 0{\cdot}4 + 1{\cdot}07$$

$$v = 1{\cdot}47\ m.$$

These instruments are constructed finer or coarser, for weaker or stronger currents of air. Very well made, complete apparatus are

constructed by Professor Recknagel, of Passau, especially a very small type, which, if fixed to a long rod, can be inserted deeply into narrow ventilating shafts, chimney flues, &c. Here the introduction and

FIG. 57.—Dynamical Anemometer, by Recknagel.

removal of the counting-works are effected very conveniently by means of a simple mechanical arrangement (Fig. 57).

Anemometers are now made for technical purposes which enable the velocity of the wind to be read off at once without calculation. The

author possesses a very sensitive instrument of this kind with aluminium paddles.

§ 115. Another kind of anemometer, applicable only in places where the instrument can be observed whilst in action, is the statical (Fig. 58). Here again the wind acts upon sails, but the tension of a watch-spring checks the rotation of the axle. The farther an index fixed on the axle is deflected upon a perpendicular annular dial-plate, the stronger is the wind; the angle of deviation is read off. If the wind is so strong that the finger index is deflected

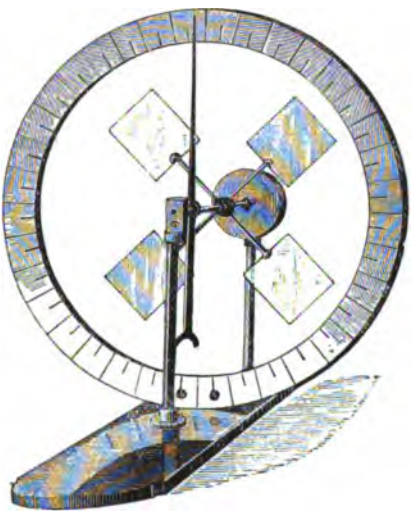

FIG. 58.—Static Anemometer.

by more than one rotation, it is pressed against a stop which indicates the uselessness of the instrument in the present case.

The calculation of the velocity of the wind in metres from the angle of deviation n is effected by the formula:—

$$v = a\sqrt{n}$$

where a is a factor which must always be empirically ascertained for each instrument (always a small proper fraction), *e.g.*, 0·06 or the like, and n is the angle of deflection in degrees.

For the determination of small velocities of wind, Recknagel's differential manometer may very well be used. To this end there is fixed to the sloping limb of the manometer a small auxiliary apparatus, which consists essentially of a round, perforated plate of brass, the perforation of which is connected with the limb of the manometer by means of a small tube and a small piece of flexible tubing. Two readings are made of the positions of the manometer, the brass plate being placed so that the wind blows one time perpendicularly upon the one side of the plate, and another time upon the opposite side. The readings are added together, and the velocity of the wind v is calculated according to the formula:—

$$v = 3{\cdot}784\sqrt[2]{\frac{w}{s}}$$

w is expressed in millimetres of water (according to § 111) by the sum of both the indications observed on the manometer; s is the specific gravity of 1 litre of air at the existing temperature and height of the barometer. See the elaborate illustrated treatise of Schönwerth (*Archiv f. Hygiene*, xi. 114).

§ 116. Anemometers are used less frequently for determining the velocity of the wind than for ascertaining the volumes of air which pass in a given time through one or several channels, apertures, &c. (determination of ventilation).

If, *e.g.*, it is required to find the volume of air which issues from a hot-air pipe it is, above all, necessary to determine the velocity of the air in the opening of the channel or duct at several points. It is general to effect successive readings of the anemometer at the places marked with a small circle (Fig. 59), to find the mean of the numbers of rotations or deflections observed, and to calculate from them the mean velocity.[1]

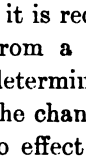

FIG. 59.

[1] The numerous measurements are necessary on account of the very different velocities which prevail in different parts of the opening. For exact investigations the same aperture must be again examined after some time. The differential manometer permits of several sectional points being examined even in narrow flues, &c., into which no anemometer can be introduced at various points.

If we know v (the velocity per second in metres), we only need to multiply with the ventilation aperture expressed in square metres to find the volume of air which enters per second in cubic metres.

Example: Let $v = 3{\cdot}5$ *m.*, and let the opening be rectangular, 76 *cm.* in width and 43 *cm.* in height; how many cubic metres of air will enter hourly?

76 and 41 *cm.* are 0·76 and 0·41 *m.*—a necessary reduction. $0{\cdot}76 \times 0{\cdot}41 \times 3{\cdot}5 = 1{\cdot}091$ *cbm.* per second, therefore $60 \times 60 \times 1{\cdot}091 = 3927{\cdot}6$ *cbm.* hourly.

The gauging of the anemometers requires especially well graduated and costly apparatus on which the anemometers can be fastened perpendicularly to the end of a long, horizontal iron rod, and turned in a circle with any desired, though constant velocity. If an anemometer is moved forwards 3 *m.* per second, this has exactly the same effect upon the mechanism as if a wind of the velocity of 3 *m.* acted upon the instrument whilst at rest. In any case an accurate gauging of an anemometer is a task which demands patience, practice, and good apparatus, and it is generally left to the maker.

Appendix: Examination of Natural Ventilation.

§ 117. The volumes of air which enter a room with closed windows through pores, fissures, and crevices are much more difficult to ascertain than those which enter through large apertures. (The action of the former constitutes natural ventilation.) We ascertain it by producing carbon dioxide in a closed space, and determine after some time to what degree the quantity of carbon dioxide found in the room is smaller than that which might be expected from the size of the room and the quantity of carbon dioxide which had been produced. This principle, indicated by Pettenkofer, is the basis of two rather distinct methods.

If at the commencement of the experiment by generating CO_2 there is produced a quantity p_1, and this quantity at the conclusion of the experiment has been reduced by natural ventilation to p_2, then (Seidel)—

$$C = 2{\cdot}303 \times m. \log \frac{p_1 - a}{p_2 - a}$$

where m is the content of the room in cubic metres, C the magnitude of ventilation in cubic metres for the time of the

experiment, log signifies the ordinary tabular logarithm, a is the proportion of CO_2 in the open air. This method presupposes, therefore, only a given increase of the proportion of CO_2 before the experiment and its determination, as well as a second determination after the experiment.

The cubic contents of the room are found by measurement and computation. The bulk of the larger articles of furniture, stoves, cupboards, &c., must be separately measured and deducted; the contents of window-niches, &c., must be added. Furniture of small bulk, tables, chairs, &c., may either be neglected, or they may be carried out of the room before the experiment.

Jacobi evolves the carbon dioxide during the experiment, and computes according to the formula (his original formula is here somewhat transformed):—

$$v = \frac{\Theta m - (k_2 - k_1)\epsilon}{\Theta (p - k)}$$

v = the *cbm.* of air which enter per hour.
ϵ = *cbm.* of air which the room contains.
Θ = number of hours of the experiment.
m = CO_2 produced in *cbm.*
k_1 = *cbm.* CO_2 in 1 *cbm* of air at the beginning of Θ.
k_2 = *cbm.* of CO_2 in 1 *cbm.* of air at the end of Θ.
k = *cbm.* of CO_2 in 1 *cbm.* of the external air.
p = true mean contents of the air in the room of CO_2 during the experiment.

This method presupposes the knowledge of a great number of factors; especially a continuous examination of the air must be undertaken by the tube method (§ 130), whilst, according to Pettenkofer and Seidel, the bottle method suffices (§ 128).

Experiments according to both methods may be combined by suitable arrangements. The production of a given quantity of carbonic acid may be best effected by burning weighed candles. 1 *grm.* stearine yields at 0° 1404 litres CO_2. The candles are placed on the floor, the air is well mixed by means of a large fan. The development of CO_2 by adding an

acid to a weighed quantity of a carbonate is faulty, because the CO_2 thus developed and not heated diffuses itself very badly in the room. Grave errors attach to both methods, as it is necessary to enter the room, when CO_2 is partly added by the breath of the operator, and partly escapes on opening the door.

Example: A small room containing 82 *cbm.*, with one window.

Two pairs of baryta-tubes with aspirators and twenty candles of the total weight of 1010 *grm.* are set up; several check analyses are always made. CO_2 determination at 8 A.M.: 0·7, 0·6, 0·7 per 1000. The candles are then lighted and let burn till 9 A.M.

CO_2 at 9 A.M.: 3·1, 3·2, 3·1, 3·3, 3·1 per 1000.

The candles still weigh 817 *grm.*; there have been therefore burnt 193 *grm.* stearine, yielding 271 litres CO_2.

CO_2 determined at 9.30 A.M.: 2·7, 2·8 per 1000.

CO_2 in the open air, 0·4 per 1000; in the corridor, 0·6, 0·6, 0·7 per 1000. Assumed average of the external air entering, 0·5 per 1000.

The two pairs of Pettenkofer baryta-tubes gave as the mean proportion of CO_2 in the air from 8 to 9 A.M. as long as the candles were burning, 2·25 and 2·35.

Calculation of the time, from 8 to 9 A.M., according to Jacobi:—

$$V = \frac{1{\cdot}0271 - (0{\cdot}0032 - 0{\cdot}007) \cdot 82}{0{\cdot}0023 - 0{\cdot}0005}$$

$$V = \frac{0{\cdot}271 - 0{\cdot}0025 \cdot 82}{0{\cdot}0018}$$

$$V = \frac{0{\cdot}271 - 0{\cdot}205}{0{\cdot}0018} = \frac{66}{1{\cdot}8} = 36{\cdot}5 \text{ cc. per hour.}$$

Calculation for the time, from 9 to 9.30, according to Seidel:—

$$C = 2{\cdot}303 \cdot 82 \cdot \log \frac{0{\cdot}0032 - 0{\cdot}0005}{0{\cdot}00275 - 0{\cdot}0005}$$

$$C = 2{\cdot}303 \cdot 82 \cdot \log \frac{27}{22{\cdot}5}$$

$$C = 2{\cdot}303 \cdot 82 \cdot \log 1{\cdot}20.$$

$$C = 2{\cdot}303 \cdot 82 \cdot 0{\cdot}079.$$

$$C = 23{\cdot}8 \text{ cc. per half hour.}$$

The natural ventilation for the first hour was therefore 36·5 *cc.*, and for the second half hour 23·8.

Hence in the room examined the air was renewed about once in two hours, when the door and the windows were closed or the former opened only for a moment.

For a more minute account see Flügge, *Untersuchungs Methoden*, p. 505, where the formulæ are deduced and the literature of the subject is quoted. Recknagel's methods for

the physical methods of determining ventilation (*Zeitschrift f. Biologie*, xv.) have hitherto been too little used in practice to be here discussed. Petri has of late improved the method, but has rendered it more complicated. As a source of carbon dioxide he uses the liquid carbonic acid of commerce (*Zeit. f. Hygiene*, vi.).

II. *CHEMICAL EXAMINATION OF THE AIR.*

§ 118. The oxygen necessary for our respiration fluctuates so little in its percentage in the air (at furthest from 20·61 to 21·0 per cent.) that it is not generally determined for hygienic purposes. Besides, its exact determination is practicable only for experienced experimentalists.[1] The nitrogen is equally constant. The air, however, contains very fluctuating and often large proportions of water and carbon dioxide, along with very small quantities of ozone, ammonia, nitric and nitrous acids. In especial cases other gases, the products of human industry, are mixed with the air, such as sulphurous acid, hydrogen sulphide, carbon monoxide, &c., which, if they occur in at all large quantities, may prove very annoying and even dangerous.

1. Determination of the Water in the Air.

A. HYGROMETERS.

§ 119. One cubic metre of air can at any given temperature contain a definite quantity of water in the state of vapour. This quantity of water (m) is called the highest possible or *maximum* moisture (at the temperature concerned). Air containing this maximum quantity of moisture at a given temperature is said to be saturated with watery vapour.

The quantity of water in grammes really present in a given case at a given temperature, is termed its absolute moisture (a). The difference between the highest possible and the absolute moisture $m-a$ is called the deficiency of saturation $=d$.

[1] In mines, caverns, &c., lower values for oxygen have been found down to 14 per cent. But such cases are too rare to require special notice.

If the proportion of the moisture really present, and the highest possible moisture, is expressed in percentages—

$$a : m = r : 100, \text{ then } r = \frac{a \cdot 100}{m}$$

the so-called *relative moisture* $100 - r = t$ is, according to Rubner, the relative dryness. Other magnitudes are not used in practical hygiene.

An expression often used, but which leads less readily to a completely clear apprehension instead of absolute and maximum moisture, speaks of absolute and maximum tension or vapour pressure. If we introduce a small quantity of water into the dry vacuum of a barometer the mercury sinks at once very slightly, because a part of the atmospheric pressure supports water instead of mercury. This sinking is only $\frac{1}{13 \cdot 6}$ of the column of water which has entered, or for 4 *mm.* only 0·3 *mm.* Then there sets in a further sinking, occasioned by the development of watery vapour in a vacuum. The higher the temperature in the vacuum so much the more water evaporates, and the pressure rises the more strongly. To each temperature there corresponds a certain tension and a certain fall of the mercury. If the water in a space containing air is evaporated the atmospheric pressure is increased by the same tension, but more slowly than in a vacuum.

It is a matter of course that also the mercurial column, the length of which serves us in the barometer as a measure of the atmospheric pressure, is supported not only by the pressure of the air, but by this pressure and the tension of the watery vapour in the atmosphere. We should, therefore, strictly speaking, always deduct 5 to 25 *mm.* tension of watery vapour from the height of the barometer if we require the atmospheric pressure alone. But this is conventionally omitted.

As it appears from Table VI., from 0° to 30° the tension expressed in millimetres of mercury almost exactly corresponds to the maximum moisture expressed in grammes, and both numbers agree from 7° to 30° (*i.e.*, in the interval which is hygienically most important), with the degrees of temperature centigrade, which is very convenient for rapid approximate mental calculations.

Those who reckon with the tension of watery vapour work with

magnitudes quite analogous to those defined above: "maximum tension," "absolute tension;" "relative tension," as the proportion of the maximum and the absolute tension. In the following considerations the percentage of water will be exclusively taken as a basis.

In order to convert statements of tension into absolute moisture the formula is used—

$$\text{absolute moisture} = \frac{\text{Tension}}{1 + 0{\cdot}00366t} \cdot 1{\cdot}06$$

and inversely,

$$\text{Tension} = \text{absolute moisture} \cdot \frac{1 + 0{\cdot}00366t}{1{\cdot}06}$$

§ 120. **Methods of Determination.**—For ascertaining the absolute moisture in the direct manner (the most accurate but circumstantial method, and hence little used), a known volume of air is drawn by means of an aspirator through two or three small flasks arranged in succession, filled with fragments of pumice steeped in sulphuric acid and weighed. The acidified pumice is obtained by heating strongly fragments of pumice in an iron capsule or Hessian crucible, or even on a wire gauze over the gas flame, throwing it whilst hot (under a draft-hood) into concentrated sulphuric acid, placing it then in a covered porcelain sieve, and after the excess of sulphuric acid has drained off, filling it quickly into the flasks. The stoppers of the flasks (each having two perforations) are either of glass, ground to fit, or of cork coated with sealing-wax. After filling, the glass connecting-tubes are closed with two flexible tube stoppers (pieces of caoutchouc tubing, into which glass rods are introduced on one side); for weighing they are taken off for a short time. If the experiment is successful all the moisture must have been retained by the first flask, whilst the second shows at most a very slight increase of weight. When the flasks have served for a few experiments II. is taken in place of I., and I. is filled afresh and serves as II.

In order to make the experiment exact a thermometer is placed both in the flask and in the air outside. If the air enters at $t°$, and if it has at this temperature the volume v, we observe in the aspirator the temperature t_1, and the volume v_1, measured by the volume of the water which has escaped, then—

$$\frac{v}{1 + a\,t} = \frac{v^1}{1 + a\,t^1}, \text{ or } v = \frac{v^1(1 + a\,t)}{(1 + a\,t^1)}, \text{ where } a = 0{\cdot}00366$$

Example:—

Temperature of the escaping air . . .	10·0°
Temperature in the aspirator	9·0°
Water escaped	20·0 litres
= Volume of air in the aspirator.	
Flask I. before experiment	50·281
Flask I. after experiment	50·442
Increase	0·161 *grm.*

Flask II. before experiment	48·102
Flask II. after experiment	48·103
Increase	0·001 *grm.*

Total increase I. + II. = 0·162.

$$v = 20{\cdot}0 . \frac{(1 + 10{\cdot}0 \times 0{\cdot}000336}{1 + 9{\cdot}0 \times 0{\cdot}000366)}$$

$$v = 20 . \frac{1{\cdot}0366}{1{\cdot}0329} = 20{\cdot}07 \text{ litres.}$$

In 20·07 litres there were 0·162 *grm.*, and therefore in 20·07 *cbm.*, 162 *grm.* In 1 *cbm.* there is 8·07 *grm.* of water, *i.e.*, the absolute moisture is 8·07. The maximum moisture at 10° is, according to the table, 9·4 *grm.*, the deficiency of saturation is therefore 9·4 – 8·07 = 1·33 *grm.*; the relative moisture is calculated at 9·4 : 8·07 = 100 : *x*; *x* = 85·8 per cent.

This method does not allow of a determination of the proportion of moisture in the air at any moment, but merely the mean proportion during the time of the experiment. It also consumes much time, and gives useful results only if carried out with great accuracy.

§ 121. More convenient methods for the determination of the absolute moisture depend upon the following fact: air which at the prevailing temperature is not fully saturated with watery vapour becomes, if successively cooled, saturated by the water present, and if refrigerated a trace lower (down to the dew-point) deposits liquid water, visible as a fine coating.

If we determine the dew-point temperature of air, we may easily read in a table the corresponding maximum moisture, which then indicates directly the absolute moisture of the air.

Daniel's hygrometer consists of an exhausted tube bent twice at right angles, and provided at each end with a ball, one of which, *A*, is gilded externally, and contains a little ether, whilst the other, *B*, is wrapped round with a piece of cloth. If ether is dropped externally upon *B*, it becomes strongly refrigerated, and the vapours of ether coming from *A* are condensed in this bulb. In the meantime fresh ether evaporates from the warmer bulb, *A*, as the internal tension is considerably decreased by the condensation of the former vapours, and thus evaporation is facilitated. The evaporation of ether in *A* consumes heat; the bulb *A* is therefore rapidly cooled, which is indicated by a small ther-

mometer sealed into it. As soon as the bulb is cooled down to the dew-point of the ambient air, water is deposited upon this bulb in minute drops, and the bright gilding appears dull.

The experiment is scarcely practicable in hot, dry air (certainly not without anhydrous ether), and it takes a long time before the dulness appears. The emission of watery vapour by the observer is very disturbing; a single incautious breath, and the dew appears. It is also presupposed that the thermometer has the exact temperature of the external surface of the bulb.

Regnault's modification of the apparatus of Daniel is an essential improvement, and can be easily improvised. Air is driven, by means of a caoutchouc ball, through a cylindrical glass vessel, coated with sheet silver for the lower third of its length, and filled with ether to

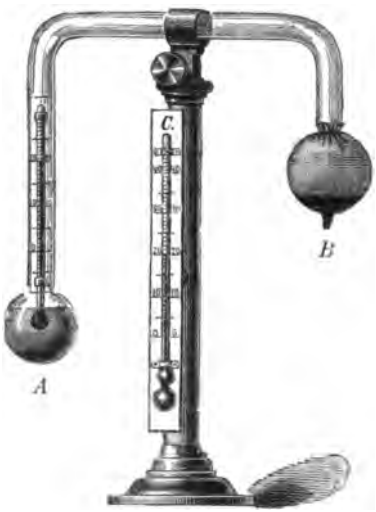

FIG. 60.—Daniel's Hygrometer.

the same height. The evaporating ether cools the portion which has remained fluid, the silver suddenly appears bedewed, and the reading of the thermometer which plunges into the ether shows at once the dew-point. In general we take the mean of the temperatures at which the dew was formed, and at which it disappeared again, as the temperature of the dew-point.

The ball can be placed at any distance and the phenomena observed through a telescope, thus obviating all the essential objections raised against Daniel's instrument, though in very hot, dry weather there is a great consumption of time and of ether. A very good light is also necessary, that the observer may recognise the very first formation of dew.

If numerous determinations have to be made in rapid succession or

almost simultaneously in different parts of a large space, this, according to the foregoing directions, is almost impracticable with dew-point hygrometers.

§ 122. For practical purposes the so-called psychrometer of August is the one most frequently used for determining the percentage of moisture in the air. It consists of two carefully-selected thermometers, alike in their movements, thoroughly verified, and graduated in tenths of a degree; one observed whilst dry and the other whilst moist. The moisture is obtained by wrapping the bulb tightly with a single layer of fine muslin, firmly tied above and below the bulb. Near the upper ligature a slender cotton wick is secured with a few stitches, and allowed to dip into a small cup of water, which supplies the loss due to evaporation. The cup of water is best placed in the manner shown by the figure. When the apparatus is first set up, the wrapped bulb and the wick are well steeped in water, and after every reading the observer must satisfy himself by touch that the muslin is really wet. The wet thermometer gradually falls by the evaporation of the water from the bulb, until its surface nearly reaches the temperature of dew-point, so that a limit is put to further evaporation owing to the air at the temperature of the wet thermometer being saturated with watery vapour. In reality the wet thermometer does not quite sink to the dew-point, because the warmer ambient air continually supplies it with heat, and this the more the greater the difference between the temperature of the wet-bulb thermometer and that of the air.

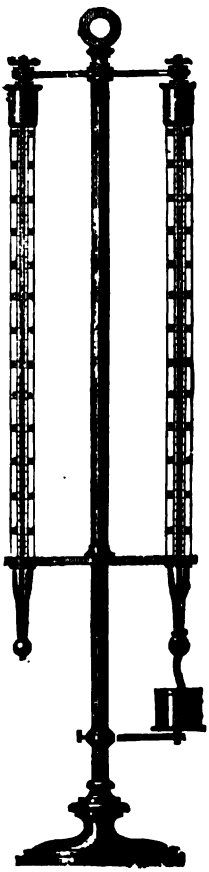

FIG. 61.—August's Psychrometer.

Let a be the absolute moisture at t_s required,

t_s = temperature of the dry thermometer,

t_f = temperature of wet thermometer,

and f the maximum moisture at temperature t_1 (as found in Table VI.).

From what has been said it is at once intelligible that the formula for calculating the absolute moisture is given from the psychrometer readings:

$$a = f - (t_s - t_f) \times c$$

where c is a constant, and for temperatures

above $0° = 0{\cdot}65$

below $0° = 0{\cdot}56$.

The formula takes into account the circumstance that t_f lies so much the higher above dew-point (and consequently f is so much greater than a), the greater is $t_s - t_f$, the difference of both thermometers. The constants, 0·56 and 0·65, are not to be regarded as absolutely exact, but depend to some extent on the height of the barometer, but more especially on the wind.

In perfectly motionless air, especially in rooms, the bulb of the wet thermometer becomes readily surrounded with a stratum of stagnant air. saturated with watery vapour, and the psychrometer ceases falling too soon; in this case the value of the constant rises up to 0·9.

If the air is saturated with watery vapour, both thermometers stand at the same height; or the moist one may be slightly the higher, as the wrapping protects it to some extent against the radiation of heat.

§ 123. If the temperature sinks below 0°, the apparatus is still capable of acting if care is taken that the layer of ice surrounding the bulb is very thin. We dispense with the wick, plunge the bulb wrapped in muslin into the water each time before reading, until any coating of ice is melted, and remove the excess of water by means of blotting-paper. The cup of water is not left in the open air, as it may be broken by the freezing of the water. The determinations of

atmospheric moisture of very low temperatures have little hygienic interest, as cold air takes up only a minimum of water.

If the absolute moisture has been determined in the manner above described, we merely require, in order to find the dew-point, to seek in the table what must be the temperature of an air which is saturated with the proportion of water ascertained.

At meteorological stations currents of air have been arranged having uniform velocities, in which the psychrometer is observed. Deneke (*Zeit. f. Hygiene*, i.) has warmly advocated the use of "sling-psychrometers" in hygienic practice, as he, even when using higher constants, did not obtain quite exact values in stagnant air with August's fixed psychrometer. The latter instrument gave the absolute moisture on an average 1 *grm.* too high.

The two thermometers are secured to plaited cords through an eye at the head, so that the measure from the beginning of the cord to the bulb of the thermometer is precisely one metre. The dry thermometer is swung round in a circle one hundred times, so that each rotation is effected in one second. The wet-bulb thermometer is then swung round in a similar manner after the bulb has been covered with a double layer of muslin, and has been plunged into water. The thermometers are read off without bringing them near the face; for the sake of distinctness the degrees of the scale are subdivided only into $\frac{1}{5}$ths, and the thermometers range only from -5 to $+40°$. Deneke obtained for his psychrometer, always working with vapour pressures, the formula:

$$f = f^1 - 0{\cdot}000706\, b(t - t^1).$$

Here f is the absolute vapour pressure required, f^1 the maximum vapour pressure at the temperature of the moist thermometer, b the reading of the barometer, t the temperature of the dry thermometer, and t^1 that of the wet thermometer.

Example 1.[1]

Temperature of dry thermometer . . .	$+15{\cdot}2°$
Temperature of wet thermometer . . .	$+12{\cdot}2°$
Difference	$3{\cdot}0°$

What is the absolute moisture?

[1] For the examples I have taken the fixed psychrometer as a basis, as it is still the most usual. For the interpolation see the instructions at the conclusion of the book.

The maximum moisture of the temperature of the moist thermometer, *i.e.*, at 12·2°, cannot be exactly seen in Table VI. At 12° it is = 10·6 *grm.*, at 15° = 11·3 *grm.* For 1° it rises by 0·7, for 0·2° it is 0·7 = 0·14 *grm.*, whence:

Maximum moisture at 12·2° = 10·6 + 0·14 = 10·7 *grm.*

Absolute moisture 10·7 − 0·65 × 3 = 8·7 *grm.*; maximum moisture at 15·2 = 12·8 + 0·2 × 0·8 = 14·4.

Deficient saturation 14·4 − 8·7 = 5·7 *grm.*

Relative moisture $14{\cdot}4 : 8 \times 7 = 100 : x$; $x = \frac{870}{14{\cdot}4} = 60{\cdot}4$ per cent.

Example 2.

Temperature of dry thermometer + 3·3°.
Temperature of wet thermometer − 1·4°.

Difference 4·7°.

Maximum moisture at − 1·4 is wanting in the table.

Maximum moisture at 0° = 4·9 *grm.*

Maximum moisture at − 2° = 4·4 *grm.*

Hence for an increase of temperature of 2° the proportion of water rises by 0·5; therefore for 0·1° $\frac{0{\cdot}5}{20}$ *grm.*

Maximum moisture at $-1{\cdot}4° = 4{\cdot}4 + 6 \times \frac{0{\cdot}5}{20} = 4{\cdot}4 + 0{\cdot}1 = 4{\cdot}5$.

Absolute moisture 4·5 − 0·56 × 4·7 = 4·5 − 2·6 = 1·9 *grm.*

Maximum moisture at 3·3° = 5·7 + 0·3 × 0·4 = 5·8 *grm.*

Deficient saturation 5·8 − 1·9 = 3·9.

Relative moisture $5{\cdot}8 : 1{\cdot}9 = 100 : x$.

Therefore $x = \frac{190}{5{\cdot}8} = 32{\cdot}7$ per cent.

§ 124. A further group of instruments determine the relative moisture directly. They depend on the property of certain fibres, animal and vegetable, to extend in length by absorbing water, and to contract on drying. The absorption of water depends not on the absolute, but purely on the relative proportion of moisture in the air.

The simplest hygrometers are those invented by Saussure, and now manufactured very perfectly by Hottinger's successors, Usteri-Reinacher of Zürich, according to Koppe's pattern.

A hair fixed at one end is loaded at the other with a weight, and runs over a wheel which carries an index. If the hair is extended in length the falling weight turns the index to the right. The index passes over a scale, the main points of which, 0 and 100, are regulated by placing the apparatus, firstly,

under a glass bell, and along with a vessel of concentrated sulphuric acid; and, secondly, by plunging it into a moist chamber, and marking the final positions of the fingers accordingly. Along with every apparatus there is supplied a sheet-metal box containing a tissue stretched over a frame. By moistening this tissue with water a damp chamber is very readily obtained. The intermediate values, which follow upon each other more closely the nearer they approach 100, are determined according to the weight method, or by means of Regnault's apparatus.

The hair must be thoroughly freed from fatty matter, and then shortened and elongated to its maximum for at least twenty times, without which preparation it does not work correctly. In every hair hygrometer the position of the point 100° should be verified from time to time by placing it in a moist place. An accompanying key serves to place the finger so that it stands at 100° in air saturated with watery vapour.

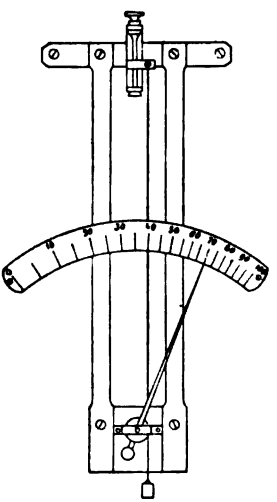

FIG. 62.—Hair Hygrometer.

Deneke found that, especially in warm and moist air, the indications of a Koppe hair hygrometer, frequently verified, are very satisfactory in their accuracy, almost as much so as those of the sling psychrometer. It is recommended to keep hair hygrometers in a space saturated with moisture, and to expose them only fifteen minutes before the intended observation.

Recently a larger series of modifications of hair hygrometers are to be met with in commerce, among which the portable but costly instruments of Schubert of Meran are especially recommended. The active part is here not a single hair, but a hygroscopic membrane.

From the indications of the hair hygrometer and from the temperature the other values are readily calculated.

Example: Temperature observed, 17°. Relative moisture, 70 per cent. What is the absolute moisture?

Maximum moisture at 17°, or according to Table = 14·5 *grm.*

$14{\cdot}5 : x = 100 : 70\ x = \frac{14{\cdot}5 \times 70}{100} = 10{\cdot}15$ = absolute moisture.

How great is the deficiency of saturation? 14·5 − 10·1 = 4·4 *grm.*

Where is the position of the dew-point? Air containing 10 *grm.* water has its dew-point at 11°, an air with 10·6 *grm.* at 12°. If the quantity of water rises by 0·6 *grm.* the dew-point rises 1°. In our case the dew-point lies at $11° + \frac{10{\cdot}1 - 10}{0{\cdot}6} = 11 + 0{\cdot}02$, *i.e.*, 11°.

B. ATMOMETERS. INSTRUMENTS FOR MEASURING EVAPORATION.

§ 125. Atmometers, apparatus intended to show how much water has evaporated from a given surface, give us a good representation of the quantity of water which an air is still able to take up. Unfortunately the existing apparatus all involve more or less important errors, so that for the present the hygienist must prefer to work with the psychrometer. We may mention:—

1. *Evaporation Boxes.* — Four-sided flat brass boxes of 1 *scm.* in surface are filled with water to the depth of a few centimetres, protected against contamination by a wire net of an open texture, and exposed in a suitable place. The quantity of water which has evaporated is determined by weighing at the beginning and end of the experiment. Each gramme or cubic centimetre represents an evaporation of the depth of $\frac{1}{10}$ *mm.* An inaccurate approximation.

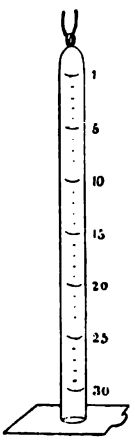

FIG. 63.—Piche's Atmometer.

Piche's Atmometer.—A glass tube closed above, and about 30 *cm.* in length and ½ to 1 *cm.* in width, is graduated into cubic centimetres, and covered at its open end with a square piece of unsized copper-engraver's paper, which has at its middle a fine aperture (prick of a needle), and closes the tube as soon as it has sucked itself full of water. The apparatus is suspended at its top by means of an eye. As water evaporates from the paper more water arrives from

the tube, and as much air penetrates into the latter as corresponds with the volume of the water which has evaporated. If the side of the square of paper measures 3 *cm.*, the water evaporates from a surface of 2 × 9 *scm.*, from which must be deducted the section of the tube which fits upon the paper. Or the section of the tube may be accounted for by leaving at one side of the paper a projecting semicircle of the size of the radius of the tube. The apparatus can only be used for comparative experiments, and it gives useful values at somewhat high temperatures. According to Riegler the water evaporates so much more readily from paper than from a surface of water that the quantities found must be divided by two in order to find the quantities which would evaporate from a surface of water under similar conditions.

C. MISTS, CLOUDS, DOWNFALL.

§ 126. Mists are formed by the refrigeration of moist air to below the dew-point; the minute drops are always formed around suspended particles of dust. (Aitkin, compare § 137.)

If mists are formed in the higher atmospheric strata we speak of clouds. Ascending currents of warm moist air occasion the formation of clouds as soon as they arrive in regions which are colder than their dew-point.

As the typical forms of clouds we distinguish:[1]—

1. *Cirrus*, the feather-cloud. Hovers at considerable heights (6 *km.* and upwards), and consists of needles of ice. In form it sometimes resembles a downy feather or a conglomerate of such feathers, or sometimes rather cotton pulled out into fine, loose stripes.

2. *Cumulus*, the heap-cloud. Mighty masses of cloud like bales of cotton, appearing brilliantly white when illuminated by the sun. They owe their origin to compact ascending currents in calm weather.

3. *Stratus*, the layer-cloud. Stripy, massive clouds, arranged in layers, produced especially when ascending atmos-

[1] The instructions for the meteorological stations in Bavaria contain very good figures of the typical forms of clouds. See Parkes' "Practical Hygiene."

pheric currents are deflected by winds. The strati are mostly at a small height above the surface of the earth (about 600 metres); they often develop into dark rain-clouds, which are distinguished by the name *nimbus*.

Between these leading forms there are many transitions: cirro-cumulus (the flock of sheep) is a formation in which cirri conglomerated more densely are gradually united into solid masses; cumulo-stratus is a common, imperfectly characteristic form of clouds, intermediate between cumulus and stratus; cirro-stratus is a diffused, semi-transparent, uniform shadowing of the heavens.

The degree of cloudiness is expressed by numbers which indicate how many tenths of the sky are beclouded. According to Mohn, the numbers may be thus interpreted:—

0 = quite cloudless,
1 = nearly cloudless,
2, 3 = lightly clouded,
4 = almost half beclouded,
5 = half beclouded,
6 = nearly overcast,
7, 8 = strongly clouded,
9 = almost entirely clouded,
10 = quite overcast.

For estimating cloudiness a freely exposed place of observation is, of course, necessary.

If the mist-globules in the cloud are increased, by the continuance of conditions favourable to their formation, to drops, rain occurs; if the process of the condensation of aqueous vapour proceeds at temperatures below 0°, there is a formation of snow (in certain cases not to be here described), or of "graupeln" (small opaque balls of snow), or of hail (conglomerates of ice and snow).

The most important meteorological observations are expressed by the following international symbols:—

Rain ●	Mist ≡
Snow ✱	Hoar-frost ⫴
Thunderstorm ϟ	Dew ○

Harvest-lightning ≷	Black-frost ⋁
Hail ▲	Vapour on hills ∞
"Graupeln" △	&c.

Wind is represented by an arrow flying in the direction of the wind, and having the more strokes in its feather the stronger it is; ⟋ is a medium and ⟋ a strong north-east wind.

D. DETERMINATION OF QUANTITIES OF DOWNFALL.

§ 127. Of late meteorologists have agreed to effect the reception of atmospheric precipitation in rain-gauges (pluviometers, ombrometers, or udometers) of the following construction.

A cylinder of sheet zinc, *a* (Fig. 64), with a large round aperture of exactly 500 *scm.*, is arranged so that the recipient opening is exactly 1 *m.* above the surface of the earth. The water flows into a sheet-metal bottle, *b*, connected by a bayonet-joint, and capable of holding 4 litres, from which it is poured into a cylindrical measure. Quantities of rain are expressed by the height in millimetres of the stratum of liquid which would be formed if all the rain were left standing. For the rain to rise to a height of 1 *mm.* over a surface of 500 *scm.*, 50 *cc.* are required. Hence we have in our bottle 50 *cc.* as often as the quantity of rain reaches 1 *mm.* The depth of rain may therefore be read off directly if we graduate a measuring-glass so that between any two marks there is exactly room for 50 *cc.* Tenths of a millimetre (*i.e.*, each 5 *cc.*) may be very conveniently read off.[1]

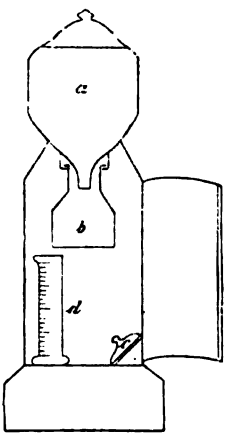

FIG. 64.—Pluviometer.

The pluviometer serves also to collect snow; the inflected

[1] The depth of downfall in millimetres shows also the quantity of rain in litres which falls on each square metre.

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upper margin is to prevent snow which has fallen in from being blown out again. After snow or hail the pluviometer is brought into the house, and the water obtained as it melts is measured. A sheet-metal cover placed over the aperture prevents evaporation. Whilst one pluviometer is read off or thawed in the house, &c., a reserve instrument is brought into action, which has been kept covered as long as the former was in use. The downfall is thus emptied and measured at 8 P.M., so that the rain is recorded, not from midnight to midnight, but from evening to evening. The date is that of the *day* of observation.

2. Determination of Carbonic Acid.

§ 128. The respiration in man and other animals, and numerous processes of oxidation, fermentation, and decomposition, occasion an intermixture of carbonic acid with the atmosphere all over the globe. In enclosed spaces inhabited by human beings the proportion of carbonic acid may become considerable, as a man at every breath expires about 500 *cc.* of 4 per cent. carbonic acid = 20 *cc.* CO_2, and artificial lights produce likewise large quantities of the same gas.

The determination, if an accurate analysis is required, is preferably effected by means of Pettenkofer's "bottle method." It depends on adding a measured quantity of baryta-water of known alkalinity to a measured volume of air. On agitation the carbonic acid combines as insoluble barium carbonate, which is quite incapable of reacting upon an indicator, and it is ascertained by titration how much less acid is now required to reach a neutral reaction than prior to the absorption of the carbonic acid.

FIG. 65.—Bottle for Baryta-water.

Preparations.—Baryta-water is obtained by dissolving

about 4½ *grm.* of pure crystalline barium hydroxide [$Ba(OH_2) + 8\,H_2O$] per litre of the water to be used. There is then further added about ½ *grm.* barium chloride per litre, for reasons stated below. The liquid thus obtained (always turbid from barium carbonate), after thorough, prolonged, and repeated shaking until everything soluble is dissolved, is allowed to settle in a suitable bottle. The stopper, as shown in Fig. 65, is provided with a twofold perforation. Baryta-water is drawn off with a long tube reaching to the bottom of the bottle, and provided at top with a flexible tube for the insertion of the pipette. The shorter tube is provided with a potash tube, to prevent air containing carbonic acid from entering in as the baryta-water is withdrawn, and thus altering the standard. The potash bottle contains fragments of pumice which have been heated in an iron dish, and thrown, whilst still hot, into strong potassa-lye, with which they are thus saturated.

We then prepare a weak solution of oxalic acid, 1 *cc.* of which saturates exactly as much barium hydroxide as ½ *cc.* CO_2, measured at 0° and 760 *mm.*; therefore 1 litre oxalic acid = ½ litre CO_2.

Oxalic acid, $\begin{matrix}COOH\\|\\COOH\end{matrix}$, always crystallises with two molecules of crystalline water,[1] $C_2O_4H_2 + 2\,H_2O$, and possesses therefore the molecular weight $2 \times 12 + 4 \times 16 + 2 \times 1 + 4 \times 1 + 2 \times 16 = 126$. CO_2 has the molecular weight $12 + 2 \times 16 = 44$. One molecule CO_2 consumes as much $Ba(OH)_2$ as one molecule oxalic acid. On saturation there are produced in one case CO_3Ba (barium carbonate); in the other $\begin{matrix}COO\\|\\COO\end{matrix}>Ba$ (barium oxalate).

1 litre CO_2 weighs at 0° and 760 *mm.* = 1·965 *grm.*

½ litre CO_2 weighs at 0° and 760 *mm.* = 0·491 *grm.*

[1] On long keeping, oxalic acid loses a little of its crystalline water—it weathers. It is recommended to recrystallise the purest commercial article once more from hot water, *i.e.*, it is redissolved in a minimum of hot water, filtered, allowed to cool, the crystals formed are collected upon blotting-paper, pressed repeatedly with blotting-paper, and allowed to lie in a warm place, in a thin layer, loosely covered, until completely dry, so that they do not adhere to smooth paper.

We have therefore the proportion—

$$44 : 126 = 0{\cdot}491 : x.$$

$x = \frac{126 \times 0{\cdot}491}{44} = 1{\cdot}404\,grm.$ oxalic acid must be dissolved in 1 litre in order that 1 *cc.* may combine with the same quantity of alkali as $\frac{1}{4}$ *cc.* CO_2.

We fill a burette with this solution, thrust a pipette of 25 *cc.* into the flexible tube of the baryta bottle, ease the pinch-cock, and slowly suck the pipette full. We reject this first quantity as being perhaps not quite clear, and allow a second pipette full to flow into a small flask holding 100 *cc.* We then add three drops of 1 per cent. alcoholic solution of rosolic acid, which turns it to a pale but distinct rose colour. We next run in oxalic acid from the burette, which has been previously filled exactly to 0, at first boldly and then more slowly, and with frequent agitation until the rose colour suddenly turns yellowish. If about 20 to 25 *cc.* of oxalic acid have been used the strength of the baryta-water is correct. If a decidedly larger quantity has been used the solution must be diluted accordingly. The titration should be effected in a room which is little used, and as far as possible the air expired by the operator should be prevented from coming in contact with the contents of the flask. It is best to work at an open window.

In titrating we must cease as soon as a yellowish colour exists for a moment the first time. On longer standing, a specimen which has been exactly titrated often turns red again, which is explained as follows:—

If we have an absolutely clear baryta-water, free from barium carbonate, a yellow colour appears as soon as the reaction $Ba(OH)_2 + C_2O_4H_2 = BaC_2O_4 + 2H_2O$ is perfectly completed, and the liquid remains yellowish in consequence of a minimum excess of free oxalic acid.

But if the baryta-water holds traces of barium carbonate in suspension, the first reaction is also, indeed, first effected, but after the yellow colour has appeared the small excess of oxalic acid acts slowly upon the traces of the $BaCO_3$, forming the neutral salt BaC_2O_4, and setting CO_2 at liberty. This CO_2 now slowly dissolves further particles of suspended $BaCO_3$ to $\begin{matrix} CO_2 / OH \\ CO_2 \backslash OH \end{matrix} > Ba$, *i.e.*, forming a barium bicarbonate soluble in water and of an alkaline reaction, which gradually reproduces the red colour.

This is the place to explain why, according to Pettenkofer's instructions, barium chloride is added to the baryta-water. The caustic baryta of commerce, in addition to $Ba(OH)_2$ and $BaCO_3$, always contains NaOH or KOH (sodium or potassium hydroxide). An addition of $BaCl_2$ converts 2NaOH into $2NaCl + Ba(OH)_2$. This addition is necessary, since sodium hydroxide may occasion errors as follows :—

There is formed from $Ba(OH)_2 + NaOH + BaCO_3$ by the addition of $C_2O_4H_2$ until a yellow colour is produced, $BaC_2O_4 + BaCO_3 + Na_2C_2O_4 +$ a trace of free oxalic acid or an acid salt.

But $Na_2C_2O_4 + BaCO_3$ are transformed to $BaC_2O_4 + Na_2CO_3$. The latter immediately saturates (not gradually, like barium carbonate in the absence of sodium oxalate) the small excess of oxalic acid, and the liquid is again alkaline. A further addition of oxalic acid reconverts the Na_2CO_3 completely into $Na_2C_2O_4$, which is again transformed with a portion of the suspended barium carbonate, so that the alkaline reaction returns as long as the last trace of suspended barium carbonate (the titration of which must be carefully avoided) is decomposed by the oxalic acid. This source of error is eliminated if every trace of barium carbonate is avoided; but, as it is so easily removed, the addition of barium chloride should never be omitted.

§ 129. After the solutions are prepared, and the standard of the baryta-water is determined, the experiment itself begins. A bottle holding about 4 litres accurately measured, clean, and dry, or, if it has not been measured, weighed when dry and empty, is filled with the ambient air by 40 to 60 blasts with a bellows, when the experimentalist must be careful that his breath does not mix with the air forced into the bottle. The bottle is therefore placed on the floor, the nozzle of the bellows is fitted with a flexible tube 50 to 60 *cm.* in length, and the operator stands erect or turned aside whilst the air is pumped in. As soon as this is done the mouth of the bottle is covered with a sound, double, well-fitting caoutchouc cap. This should be done at once, though not in anxious haste. The temperature of the room and the state of the barometer and its level are then read off.

Baryta-water is then sucked up out of the store bottle into a pipette holding 100 *cc.*; the latter is inserted into the bottle as deep as possible (partially easing the caoutchouc cap), and the baryta-water is let flow in. The pipette is then closed at top with the finger, it is warmed by grasping in the hand, and the last drops of baryta-water are thus driven out. The pipette is drawn back and the cap is closed again.

The bottle is then inclined and caused to revolve gently on its longitudinal axis about three times every five to ten minutes, with similar intervals of rest, so as to wet the sides of the bottle. Care must be taken not to project the liquid against the cap.

When the absorption is completed, after 30 to 45 minutes, the liquid, which is now rendered turbid by barium carbonate, is quickly poured—preferably at an open window or in the open air—into a small bottle holding about 100 *cc.*, which is then allowed to stand at rest for some hours, closed with a well-ground stopper until the precipitate has completely subsided. Of the clear supernatant liquid 25 *cc.* are quietly drawn off by means of a pipette introduced almost to the sediment at the bottom. Above all, the suction must not be interrupted for a moment lest the precipitate should be stirred up by the reflux of the liquid. For momentarily closing the pipette the point of the tongue may be advantageously used (see § 24). These 25 *cc.* are titrated at the open window, as recommended above, and for safety's sake the titration is repeated upon a second portion.

If the bottle had not been measured, but only weighed when empty and dry, it is now filled exactly to the top with water and weighed again. The difference of the weights gives the volume of the bottle, from which 100 *cc.* must be deducted, as they were occupied with baryta-water. As the calculation of the titration (see below) shows us the volume of the CO_2 at 0° and 760 *mm.*, we must also reduce to the above magnitudes the volume of the air which we determined at $t°$ and b*mm.* (height of the barometer reduced to 0°).

This is effected according to the following considerations: All gases expand when heated according to the formula $V_t = V_0 (1 + a t)$ where—

V_0 = volume at 0°.

V_t = volume at $t°$.

a a constant = 0·00366, or

$$V_0 = \frac{V_t}{1 + a t}$$

Further, the volume of all gases is inversely as the pressure to which they are subjected. If

V_b is the volume at pressure b,

$V_{b'}$ the volume at pressure b',

then $V_b : V_{b'} = b' : b$, or $V_b = \frac{V_{b'} \times b'}{b}$ and $V_{b'} = \frac{V_b \times b}{b'}$

If, therefore, an observation has been made at temperature t, and at the height of the barometer b; and if the volume V_{tb} must be reduced to V_{0760}, *i.e.*, to the volume at 0° and 760 *mm.*, this is effected according to the simple formula—

$$V_{0760} = \frac{Vtb \times b}{(1 + at) \times 760}$$

Example. Weight of empty bottle, ascertained at commencement of the experiment . . . . . 4050 *grm.*

Weight of full bottle, ascertained at end of experiment . . . . . 8050 *grm.*

4000 *cc.* = vol. of bottle.

Height of barometer during experiment, 740 *mm.* at 17°, *i.e.* = 738 at 0°.

Temperature of air at point where taken, 25·0°.

Volume of bottle = 100 *cc.* baryta-water at 25° = 3900 *cc.*

Volume of bottle = *cc.* baryta-water at 0° and 760 barometer = $\frac{3900 \times 738}{(1 + 0{\cdot}00366 \times 25) \times 760} = 3469.$

25 *cc.* baryta-water consumed before the experiment 24·0 *cc.* oxalic acid.

25 *cc.* baryta-water consumed after the experiment 21·5 *cc.* oxalic acid.

Therefore in 25 *cc.* baryta-water there is saturated by CO_2 a quantity corresponding to 2·5 *cc.* oxalic acid = $\frac{2{\cdot}5}{4}$ *cc.* carbonic acid.

Hence 100 *cc.* baryta-water absorbed $4 \times \frac{2{\cdot}5}{4} = 2{\cdot}5$ *cc.* CO_2.

3469 *cc.* of air at 0° and 760 *mm.* contain 2·5 *cc.* CO_2 at the above temperature and pressure, *i.e.*,

$$3469 : 2{\cdot}5 = 1000 : x = \frac{2500}{3469} = 0{\cdot}72 \text{ per thousand.}$$

In order to facilitate these calculations various tables have been constructed, of which by far the most convenient are those of Baumann (*Tafeln zur Gasometrie*, Munich, 1885), which enable $\frac{b}{760(1 + 0{\cdot}00366 \times t)}$ to be read off directly for all pressures of the barometer from 640 to 780 *mm.*, and

from $-2°$ to $+32°$. This cheap and practical little book contains a number of other convenient and useful tables.

Uffelmann's recently proposed modifications for the determination of CO_2 (*Archiv f. Hygiene*, viii.) are scarcely improvements or facilitations. It is interesting that titration in turbid baryta-water, after the barium carbonate has stood for twenty-four hours, scarcely occasions an error, as apparently only recently precipitated $BaCO_3$ is decomposed by oxalic acid.

Bitter (*Zeit. f. Hygiene*, ix.) has again very closely studied the method of Pettenkofer, and for unusually exact experiments he has introduced some improvements of minor importance. (Titration in the absorption vessel itself, use of strontia-water, phenolphthalein, &c.)

§ 130. If it is required to ascertain the mean proportion of carbonic acid in air during a considerable time, or to examine the air in places which are not readily accessible according to the method just described, we proceed as follows (Fig. 66):—

The air which is taken by means of a tube at the place desired is caused by means of the aspirator *A* to pass slowly in a current regulated by the pinch-cock in small but rapidly successive bubbles through the inclined Pettenkofer tubes, filled with baryta-water, whereby the carbonic acid is absorbed. The smaller Pettenkofer tubes contain generally 100 *cc.*, the larger ones 250; two tubes should always be fixed one behind the other. The smaller tubes are generally sufficient, but it is often advisable, *e.g.*, in examining ground air, &c., to use stronger baryta-water, about 10 *grm.* barium hydroxide per litre. The oxalic acid may also be made of double strength, *i.e.*, 1 *cc.* = ½ *cc.* CO_2, or 2·808 *grm.* per litre.

For setting the apparatus to work, the baryta-water, measured exactly, is first filled into the tubes; the caoutchouc plug with the open glass tube is inserted, the bulb is connected with the aspirator, and, lastly, the tube in the caoutchouc stopper is connected with the tube for drawing the sample. The slope of the tube is then so arranged that the bulb and the adjacent part of the tube are not filled with the baryta-water; the pinch-cock on the efflux tube of the aspirator is cautiously opened, and whilst the air passes through the

baryta-water in a continuous series of fine bubbles, the effluent water of the aspirator is allowed to flow into measures or weighing glasses placed below.[1]

If the pipe leading to the baryta tube is long, the aspirator may conveniently be allowed to work for a time without introducing the absorption tube, so as to fill the apparatus with the air to be examined. In general, two experiments are made by this method in succession.

If the baryta-water appears sufficiently turbid after 1 to 4 litres of air have been passed through, the spring clip of the

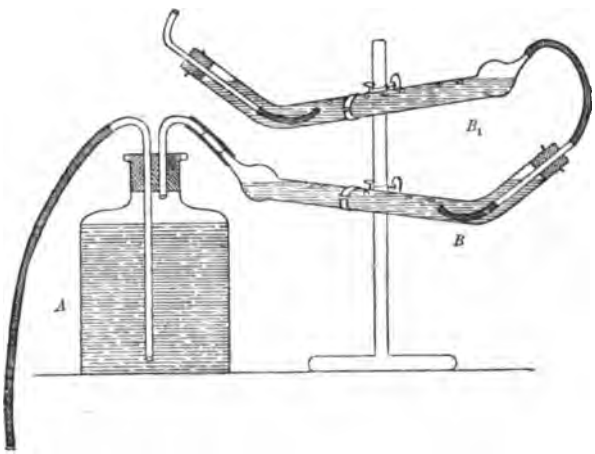


FIG. 66.—Determination of Carbonic Acid with Pettenkofer's Tubes.

aspirator is closed, the caoutchouc stopper of the first tube is cautiously withdrawn, the aperture is closed with the thumb of the right hand, the flexible pipe is taken off the beak of the tube, then the screw which supports the baryta tube, and the contents of the tube are quickly poured through the beak (after the removal of the thumb) into a stoppered bottle, in which it is allowed to deposit. The titration is then effected as above. The volume of the water which has flowed

[1] The fine distribution of the air is effected by fixing a piece of flexible piping on the small glass tube which traverses the stopper, dips into the baryta-water above the bend, and terminates just below the level of the liquid.

out shows the volume of the air which has been drawn through at the temperature in the bottle, which is generally assumed as equal to the temperature of the air entering (for correction see § 120). By means of this number and the height of the thermometer (reduced to 0°) the volume of air examined is calculated at 0° as in the bottle method, and thus its proportion of carbonic acid is ascertained.

Example. Suppose that at 10° and 720 (reduced to 0°) barometric pressure 2000 *cc.* of air have been drawn through the tubes, the first tube containing 150 and the second 100 *cc.* of baryta-water. The standard of the baryta-water was determined before the experiment: 25 *cc.* correspond to 27 *cc.* oxalic acid solution (1 *cc.* oxalic acid solution = $\frac{1}{2}$ *cc.* carbonic acid). After the air had passed through there were in tube 1 for 25 *cc.* still 18 *cc.* of oxalic acid necessary, in tube 2 for 25 *cc.* there were still needed 25 *cc.* oxalic acid. Hence $6 \times (27 - 18) = 54$ were used in the first, and $4 \times (27 - 25) = 8$ in the second; in all, 62 *cc.* oxalic acid less used, or $\frac{62}{2} = 31$ *cc.* CO_2 were absorbed. The reduced volume of the air is 1828 *cc.*; the proportion of carbonic acid 1·68 per thousand.

§ 131. For approximate determinations — sufficient for most cases in hygienic practice — so-called "minimetric methods" have been elaborated. Angus Smith, and subsequently Lunge, endeavoured to determine the proportion of CO_2 from the quantity of air necessary for producing a certain degree of turbidity in clear baryta-water.

For this purpose Lunge forces air from a caoutchouc ball containing exactly 50 *cc.* through a volume of baryta-water until a mark (a pencil cross upon paper) can no longer be distinguished through a stratum of baryta-water, always of equal thickness. The apparatus has not gained many friends; a distinct final reaction was never obtained, and a given turbidity was considered as sufficient, too strong or too slight, according to the illumination or the observer's sharpness of vision.

Recently Lunge and Zeckendorf have modified the method in an excellent manner, so that it may now be recommended whenever a rapid determination of carbonic acid is required without any necessity for absolute accuracy. A condition of the practical utility of the method is that the proportion of CO_2 in the air examined must not be too small, not under one part per thousand, as the experiments are otherwise too tedious.

Lunge prepares a decinormal solution of soda (5·3 *grm.* anhydrous carbonate, or 14·3 *grm.* of soda crystals with 10 molecules of crystalline water), are dissolved to 1 litre, in which 0·1 *grm.* of solid phenolphthaleine is dissolved, forming a purple solution which keeps for months in a well-stoppered bottle.

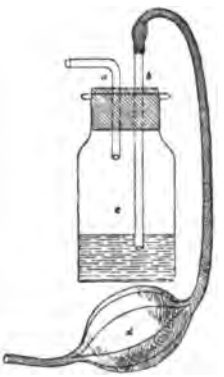


FIG. 67.—Apparatus for Lunge-Zeckendorf's Minimetric Method.

Of this solution 2 *cc.* are added to 100 *cc.* of distilled, boiled out and refrigerated water, which is daily prepared afresh for every series of experiments. Water is boiled in a flask, and after prolonged boiling, when only large bubbles rise, well stoppered with caoutchouc and allowed slowly to cool. After the apparatus (Fig. 67) in its empty state has been filled with the air in question by repeated compression and relaxation of the caoutchouc bag (*d*), 10 *cc.* of the dilute reagent are introduced, the contents of the bag (70 *cc.*) are slowly pressed through the liquid and shaken for one minute after the flexible tube has been compressed with the fingers. Meantime the bag is filled anew and air is forced in, and the bottle each time shaken for a minute until the violet colour has changed to a yellow.

Lunge gives the following table for the use of $\frac{1}{300}$ normal soda solution:—

	Per 1000.		Per 1000.		Per 1000.
48 Fillings	= 0·3	13 Fillings	= 0·8	6 Fillings	= 1·5
35 ,,	= 0·4	10 ,,	= 0·9	5 ,,	= 1·8
27 ,,	= 0·5	9 ,,	= 1·0	4 ,,	= 2·1
21 ,,	= 0·6	8 ,,	= 1·2	3 ,,	= 2·5
17 ,,	= 0·7	7 ,,	= 1·4	2 ,,	= 3·0

Dr. Fuchs, who examined the entire method scrupulously by my direction, and compared it with the bottle method, found, as it was to be expected, that this weak solution gave too uncertain results in impure air, since a single bagful, or only a fraction of it, often produced decoloration. He

obtained very good results with a solution of double strength, 4 *cc.* of the original soda solution to 100 *cc.* of water, approximately as follows:—

16 Fillings	= 1·2 per 1000.	5 Fillings	= 3·0 per 1000.
8 ,,	= 2 ,,	4 ,,	= 3·6 ,,
7 ,,	= 2·2 ,,	3 ,,	= 4·2 ,,
6 ,,	= 2·5 ,,	2 ,,	= 4·9 ,,

For air which is only slightly impure (in well-ventilated rooms with few inmates), the weak solution must be recommended, and in worse hygienic conditions the stronger liquid. Such determinations are admissible only, for practical purposes, where errors up to 10 per cent. of the total do not seriously compromise the result; but in the hands of the school physician, the factory inspector, &c., they do very good service. For details see Lunge and Zeckendorf, *Zeit. f. angewandte Chemie*, 1888, H. 14, and 1889, H. 1.

The Lunge-Zeckendorf method has been recently much improved by Rosenthal. He does not force the air through the liquid with a ball, but he sucks air by means of a tube finely drawn out through a measured volume of solution of phenolphthaleine in soda until decoloration exactly ensues. I can strongly recommend the method. The aspiration apparatus consists of two glasses holding ½ litre each, connected by a flexible tube, which are filled alternately. A fine glass syphon allows the current of air to be made as slender as it may be required. For the determination of CO_2 the current of air must be very slow; the bubbles of gas drawn through must be very small, in order that perfect absorption may ensue. I have also found the apparatus useful in various other gas analytical operations. (Description and illustration in *Schulz Münchner Med. Wochenschrift*, 1891.)

The inverse process of adding to a measured volume of air a solution of soda coloured with phenolphthaleine (shaking frequently) until a permanent red colour appears has been proposed by Nienstädt and Ballo. Bitter has modified the process, and finds it practicable. In comparison with the apparatus of Lunge-Zeckendorf and Nienstädt-Ballo's, that

of Rosenthal has the defect of being less portable, as it fills a rather large box.

3. Detection and Determination in Air of Chemical Constituents which are Present only in Small Quantities.

§ 132. Concerning the substances present in the open air in small quantities, and as yet partly unknown and concerning their hygienic meaning, the following indications must suffice (particulars about ozone in Flügge, *Lehrbuch der Hyg. Untersuchungs-methoden*, 1881).

1. **Ozone** = O_3. An oxygen molecule of three atoms, which readily passes into ordinary oxygen (O_2) by splitting off one atom of free oxygen, possessing very great oxidising energy. Not only its qualitative detection, but a method for its quantitative determination, were formerly founded upon its property of liberating iodine from potassium iodide:—

$$2KI + H_2O + O_3 = 2KOH + O_2 + I_2.$$

Potassium-iodide starch papers (formed by dipping slips of filter-paper in starch in which a little potassium iodide had been dissolved) were suspended in the space to be examined, and from their change of colour in a certain time the quantity of ozone was estimated, the blue colour of the paper becoming darker by the formation of starch-iodide the more iodide was liberated. But even if we eliminate, according to Wolffhügel's (*Zeit. f. Biol.*, ii.) advice, the more serious errors of this method (in windy weather more air passes over the paper than in a calm; sunlight bleaches the blued paper), and proceed by drawing a measured quantity of the air in question over potassium-iodide starch paper in the absence of light, and then compare the colour of the moistened paper with a scale, we obtain no exact results. Hydrogen peroxide (H_2O_2) and nitrous acid (N_2O_3), which are always present, as well as chlorine, which occurs less commonly, and volatile organic acids, produce the same blue colour, whi'st hydrogen sulphide

and sulphurous acid act in the opposite direction, or destroy the blue colour. The moisture in the air is not indifferent, since the paper turns blue more readily in moist than in dry air. The detection of ozone by means of thallous oxide paper is rather better, but this reaction is common to O_3 and to H_2O_2.

To distinguish O_3 from N_2O_3 we use, along with the potassium-iodide starch paper, slips of litmus paper, perfectly neutral (violet), and saturated with solution of potassium iodide. The latter turns blue (by the formation of KOH) only in presence of ozone; N_2O_3, Cl, &c., redden or bleach the colour, or leave it unchanged.

Concerning hydrogen peroxide (H_2O_2) in air there exist hitherto no investigations on the part of hygienists. It is never absent in air, and it may be detected especially in rain, hail, and snow. It also reddens potassium iodide paste, but, except on the addition of ferrous sulphate, very slowly, only in the course of some hours—a contrast to ozone. A good qualitative reaction is the following:—

To the liquid containing H_2O_2 there is added a drop of a 1 per cent. solution of potassium chromate, a little ether, and a few drops of dilute sulphuric acid. The ether, on shaking, takes the splendid blue colour of perchromic acid even if but little H_2O_2 is present. The smaller the quantity of this compound present the less potassium chromate must be used. According to the researches of Schöne (*Ber. der Deutsch. Chem. Gesell.*, xi., xiii., xvii.), the majority of the former statements concerning ozone refer to hydrogen peroxide. Hitherto no reaction has succeeded with atmospheric air which can be solely due to ozone.

Ammonia, nitric and nitrous acid, are constantly found everywhere in the open air, especially in the strata of the atmosphere nearest to the ground, but in such minute and hygienically insignificant quantities that we cannot here touch upon the methods for their determination. Sulphurous acid, produced by the combustion of fuel, can generally be found in the air of cities (see § 135), most distinctly on examining snow. Sendtner found in 1 *kilo.* of recent snow 7 *mgrm.* sulphuric acid, and in old snow as much as 91 *mgrm.* The sulphurous and sulphuric acids have here been jointly determined as sulphuric acid; in reality from 50 to 95 per cent. is present as sulphuric acid, and the rest as sulphurous acid.

The examination for gases of hygienic importance is much more essential in the air of work-rooms, factories, &c., than in the open air.

§ 133. **Organic Matter.**—Recently Uffelmann (*Arch. f. Hygiene*, viii.) has proposed a method for determining organic

matter in the air, by which he has obtained very useful results. He distinguishes organic matter as pulverulent and gaseous: the former he filters off by means of glass wool; the latter is absorbed by 10 *cc.* of a very dilute solution of potassium permanganate (1 *cc.* representing 0·0787 *mgrm.* oxalic acid, and yields 0·01 *mgrm.* oxygen). The matter filtered out at the end of the experiment is boiled with dilute solution of permanganate, and both the permanganate solutions are then titrated with oxalic acid (1 *cc.* = 0·0787 *mgrm.* oxalic acid); the experiment is conducted in the same way as in the determination of organic matter in water.

The method suffers from grave defects; among others the organic particles of dust are of little interest, and are very unequally attacked by permanganate; the organic gases are also very imperfectly taken up by the cold, highly diluted solution of permanganate; from 10 to 20 litres of air must be passed through in from two to four hours in order to obtain appreciable results, and "an excess or deficiency of 0·05 *cc.* of solution of oxalic acid may make the result too high or low by 20 to 30 per cent." As a preliminary indication until better procedures are found this method may be employed, but in the meantime it is scarcely fit for practical use.

Since this criticism was written in the last German edition of the work, Nekam, working in Fodor's laboratory, has raised similar objections founded upon experiments, and has especially referred to the spontaneous decomposition of the permanganate solution (*Arch. f. Hygiene*, xi.). Archarow (*Arch. f. Hygiene*, xiii.) has somewhat modified the method. He takes the air-bubbles very small, introduces three permanganate tubes, one behind another, heated to 40°, uses the permanganate and the oxalic acid five times more strongly diluted than does Uffelmann, the solution of permanganate is acidulated, and the spontaneous decomposition is taken into account. Archarow, like Uffelmann, alleges that he has found distinct differences between fresh air and that of the laboratory. But even yet the method scarcely seems fit for practical use.

Our knowledge of the several organic matters excreted by man from the skin and the lungs is still very imperfect, the

alleged volatile alkaloid in the exhaled air (Würtz, Brown-Séquard, and d'Arsonval) has not yet been satisfactorily demonstrated; only small quantities of ammonia have been traced. From sweat there pass into the air small quantities of fatty acids and ammonia; from the intestinal gases, beside hydrogen and hydrocarbons, traces of fatty acids, hydrogen sulphide, methylmercaptan, and skatol.

§ 134. **Carbon Monoxide,** CO.—The detection depends on the property of hæmoglobine to form with carbon monoxide a compound of characteristic attributes.

Demonstration according to Vogel.—Into a capacious bottle, holding preferably about 10 litres, we force air from the room to be examined; we add 10 *cc.* of a solution of blood diluted with 300 parts of water until the colour is of a pale red. The bottle is closed with a caoutchouc cap, and made to revolve gently for thirty minutes without shaking, as in the determination of carbon dioxide, and the liquid is then poured into a test-glass. If CO is present a bluish shade often appears at once, on comparison with a check specimen of the original blood, which is very characteristic. The two specimens of blood are then examined with the spectroscope.

If the proportion of CO is large we may perceive, without further addition, if the apparatus has a scale or comparative prism, that each specimen displays two bands, but that the left-hand band of the CO-hæmoglobine, situate in the yellow, lies rather nearer to the right than in the oxygen-hæmoglobine. If we now add to each solution two drops of yellow ammonium sulphide, or of Stokes's liquid (ferrous-ammonium tartrate),[1] the two bands in the oxygen-hæmoglobine disappear, and in their place there is produced a broad band of reduced hæmoglobine with indefinite outlines. In the carbon monoxide hæmoglobine both the bands remain, as this compound resists reduction. In this manner 2½ per cent. of carbon monoxide may be recognised in air.

[1] *Stokes's liquid.* Dissolve ferrous-sulphate in water, add solid tartaric acid until a strong precipitate is produced, and redissolve it in an excess of ammonia so as to form a blackish green liquid.

The condition for the success of the experiment is a sufficient dilution of the blood. If the solution is too concentrated in the specimen containing carbon monoxide, so large a quantity of reduced hæmoglobine is produced by the reduction agents that the interval between the bands appears filled up. At least 28 per cent. of the existing hæmoglobine must be combined with carbon monoxide to render the latter recognisable.

To make the process more sensitive (*i.e.*, to show $\frac{1}{2}$ *cc.* carbon monoxide in 10 litres of air, or 0·05 per thousand), Fodor (*Deutsche Vierteljahrsschrift f. oeffent. Gesundheitspflege*, xii.) has rendered it more complicated. It must be objected that his method depends on the doubtful principle of recognising CO by the black precipitate of metallic palladium which it produces in a solution of palladious chloride, whilst various other substances which can only be in part excluded by the ingenious arrangement of the experiment, *i.e.*, acetylene and other hydrocarbons, react in a similar manner.

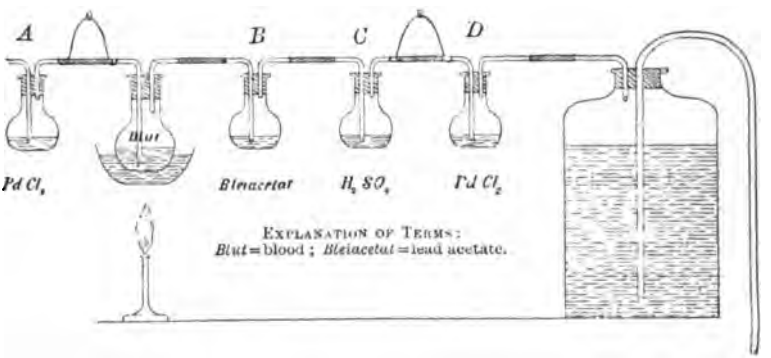


FIG. 68.—Detection of Carbon Monoxide according to Fodor.

A large bottle (10 litres) is filled with the suspected air, the CO is absorbed as above in dilute blood, and the apparatus here figured is set in action by opening the clip-screw of the aspirator and heating the water-bath in which the diluted blood is placed. The current of air, which is freed from all substances capable of reducing palladium by passing through $PdCl_2$ (flask *D* takes along with it various gases from the heated blood). The flask *B*, containing lead acetate, arrests any sulphuretted hydrogen; the flask *C*, charged with sulphuric acid, keeps back any ammonia, both of which might occasion precipitates in $PdCl_2$. Any CO passes *B* and *C*, and comes into action in the $PdCl_2$ (flask *D*), when it occasions a coloration of the yellow liquid, which is first blackish brown and then black, whilst there is formed a dark deposit of metallic palladium.

Recently Welzel with Kunkel has developed a very simple method, which is at least as effective as that of Vogel, whilst

it does not require a spectroscope.[1] This, therefore, deserves to be the method for practice.

If we absorb the carbon monoxide of a volume of about 10 litres of air in 20 *cc.* of a 20 per cent. solution of blood as above, and if we mix this blood, and also a check solution with various substances capable of precipitating albumen, we obtain precipitates of different colours. They are brownish in each solution, but in the carbon monoxide blood they incline more to a brownish reddish, and in the check specimen to a yellow or a grey.

Welzel recommends as the best among the many reactions which have been investigated (the colorations mentioned refer to a small proportion of CO):—

1. To 5 *cc.* of the blood solution we add 15 *cc.* of a 1 per cent. solution of tannin and shake it up, whereby a precipitate is formed. The turbidity subsides slowly; after one to two hours the incipient difference of colour is very distinct, but more so in from twenty-four to forty-eight hours. In blood containing carbon monoxide it is brownish red, but in ordinary blood of a greyish brown. After ten months this difference of colours still exists if it is kept in bottles closed with caoutchouc stoppers.

2. To 10 *cc.* of the solution of blood we add 5 *cc.* of a 20 per cent. solution of potassium ferro-cyanide and 1 *cc.* acetic acid (1 volume glacial acetic acid + 2 volumes water.) The precipitate in the blood containing carbon monoxide very soon becomes reddish brown; but in oxygen-hæmoglobine a greyish brown. This difference decreases in thirty minutes, and it disappears in from two to six days. By means of both these processes Welzel has demonstrated 0·023 per thousand, *i.e.*, $\frac{1}{4}$ *cc.* of carbon monoxide in 11 litres of air.

Quantitative determinations of carbon monoxide in air are difficult, and can be effected only if the proportion is not too small. (See Sudakoff, *Arch. f. Hygiene*, v.)

Carbon monoxide in the air is often derived from coal gas,

[1] Welzel, *Ueber den Nachweis des Kohlen ozydhæmoglobins. Verh. der Phys. Med. Gesellsch. in Würzburg*, xxiii. No. 8, 1889. A full bibliography of the subject.

in which it is hygienically the most important and significant substance (see § 444). Carbon monoxide is also easily recognised in the smoke of cigars.

§ 135. **Preliminary Remarks.**—All the gases mentioned in this paragraph are absorbed from the air by means of suitable absorbments placed in glass vessels, among which the pear-shaped bulb of Will-Varrentrapp (Fig. 69) and Peligot's tube (Fig. 70) are preferred. Two such apparatus are connected in succession. Latterly I have preferred as absorbent vessels long, solid test-glasses, into which dips a fine glass tube drawn out. We obtain in this manner smaller bubbles and more complete absorption. I have generally found a second absorption apparatus unnecessary. The

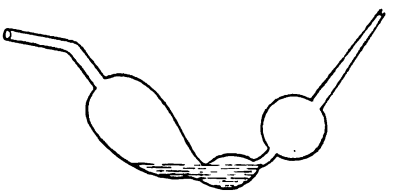


FIG. 69.—Will-Varrentrapp's Pear Tube.

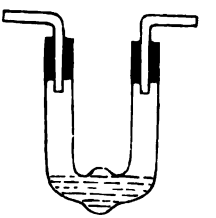


FIG. 70.—Peligot's Tube.

extraction of the air is effected by means of aspiration, or with Rosenthal's carbonic acid apparatus (§ 131). After the end of the experiment the absorption apparatus is emptied into a beaker glass and washed out two or three times with water, and the quantity is determined by simple titration. Table VII. allows of the conversion of the milligrammes of gas found into cubic centimetres, and the expression of the quantity per thousand, the air being reduced to a pressure of 760 *mm.*, and to the temperature of 0°, according to § 129. For a qualitative recognition, if nothing is especially remarked, the sense of smell and the exposure of moist slips of test-paper generally suffice, or we select a method connected with the quantitative determination.

Hydrochloric Acid Gas (HCl.)—Absorption in 5 to 10 per cent. soda-lye, which must be free from sodium chloride. The

contents of the vessel must be exactly neutralised with dilute nitric acid free from chlorine, testing with a small slip of litmus paper. The neutral solution of sodium chloride thus obtained is titrated with centinormal solution of silver nitrate. (See *Water Analysis.*)

Both apparatus are to be filled rather fuller than they are here figured. If the liquid to be titrated is very poor in HCl, I have found it better to add an exact quantity, say 10 *cc.* of a solution of sodium chloride, which alone consumes 10 *cc.* of the silver solution, and to calculate the HCl which has been absorbed from the increase.

Sulphurous Acid (SO_2).—Absorption liquid potassa-lye at 20 per cent. The titration must be afterwards effected as rapidly as possible with permanganate (Mori-Pettenkofer, *Arch. f. Hygiene*, ii.), or, more conveniently, by absorption in solution of iodine and titration with sodium hyposulphite (thiosulphate). The last method alone will be here described:—

Necessary Solutions.—(1.) Decinormal sodium hyposulphite = sodium thiosulphate. $Na_2S_2O_3 + 5H_2O$ is the formula of this finely crystallising salt. 248 its molecular weight, 24·8 *grm.* yield 1 litre of the decinormal solution; in iodometry 1 *cc.* of a normal solution must correspond to 1 *cc.* of a normal solution of iodine.

(2.) Decinormal solution of iodine, *i.e.*, 12·65 *grm.* iodine per litre.

The former solution can be obtained directly by weighing out and dissolving the sodium hyposulphite. But not so the iodine solution, as iodine is never absolutely pure, and is also somewhat volatile on weighing. We therefore dissolve about 13 *grm.* iodine, rubbing it up in a little water with 20 to 25 potassium iodide, dilute to 1 litre, and diluting it still further until 10 *cc.* of the solution of sodium hyposulphite exactly suffice to decolorise 10 *cc.* of the iodine solution, which had been coloured blue by the addition of a few drops of solution of starch. The reaction is according to the formula:—

$$2Na_2S_2O_3 + 2I = Na_2S_4O_6 + 2NaI,$$

i.e., 248 *grm.* $Na_2S_2O_3$ decolorise exactly 126·5 *grm.*

iodine, forming colourless sodium tetrathionate and sodium iodide.

Conduct of the Experiment.—In the absorbent solution of iodine the reaction ensues:—

$$SO_2 + I_2 + 2H_2O = SO_4H_2 + 2HI.$$

To prevent iodine vapour from being carried away from the iodine solution and remaining undetermined, a Peligot tube is introduced after the iodine tube and filled with 5 *cc.* of decinormal, or $\frac{1}{30}$th normal solution of sodium hyposulphite, thus keeping back all the iodine. If we have used 20 *cc.* of the iodine solution, and 5 *cc.* of the hyposulphite solution, this is poured together before titration, calculating as if 15 *cc.* of the iodine solution had been used.

Sixty-four milligrammes sulphurous acid convert exactly 253 *mgrm.* iodine into hydrogen iodide, or 3·2 *mgrm.* decolorise exactly 1 *cc.* of a decinormal solution of iodine. As often, therefore, as the standard of the absorbing decinormal solution of iodine titrated with decinormal hyposulphite decreases by 1 *cc.*, so many times 3·2 *mgrm.* sulphurous acid are present in the air.

Hydrogen Sulphide (H_2S) (sulphuretted hydrogen) is recognised by the browning or blackening of slips of paper steeped in lead acetate (formation of black lead sulphide, PbS), or by the violet coloration of a slip moistened with solution of sodium nitro-prusside and weak soda-lye.

For quantitative determinations it is preferable, according to my experience, to absorb the hydrogen sulphide in a measured volume of decinormal, or $\frac{1}{30}$th normal solution of iodine, determining the reduction of the standard by titration with decinormal or $\frac{1}{30}$th normal hyposulphite. For obtaining the solutions, and for the necessary precautions, see Sulphurous Acid. The reaction is—

$$I_2 + H_2S = 2HI + S.$$

One cubic centimetre of decinormal solution of iodine contains 12·65 *mgrm.* iodine, and decomposes 1·7 *mgrm.* sulphuretted hydrogen; each cubic centimetre of sodium

hyposulphite less that we use after absorption than for the titration of the same volume of the original solution of iodine, signifies therefore the absorption of 1·7 *mgrm.* sulphuretted hydrogen.

The vapour of *carbon disulphide* (CS_2) is easily determined quantitatively. The air is drawn through absorption vessels filled with a strong solution of potassa in alcohol at 96 per cent. The CS_2 is completely taken up as potassium xanthogenate $CS\begin{matrix}/OC_2H_5\\\backslash SK.\end{matrix}$ The latter may be very accurately determined by Gastine's method, which I have often verified and applied with much success. The contents of the absorption vessels are acidulated with a little acetic acid after rinsing them out with a mixture of alcohol and water; a little calcium carbonate is added in order to neutralise any excess of acid. To the entire slightly acid solution there is added a little solution of starch, and as much water as the alcoholic potassa which has been used. A solution of iodine in potassium iodide (1·662 iodine per litre) is then added until a faint blue colour appears. 1 *cc.* of solution of iodine corresponds to 1 *mgrm.* CS_2. The equation is—

$$2CS\begin{matrix}/OC_2H_5\\\backslash SH\end{matrix} + I_2 = \begin{matrix}S-CS-O-C_2H_5\\|\\S-CS-O-C_2H_5\end{matrix} + 2HI$$

xanthogenic acid + iodine = xanthogen persulphide + hydrogen iodide.

$76CS_2$ corresponds to 126·5 iodine, and therefore $1CS_2$ = 1·662 iodine.

Nitrous Acid (N_2O_3) may be absorbed like SO_2 in pure potassa-lye, and likewise titrated with potassium permanganate. See § 197.

Chlorine and Bromine.—Absorb in 15 to 20 *cc.* of a pure 10 per cent. colourless solution of potassium iodide. Each molecule of chlorine or bromine sets free a molecule of iodine—

$$Cl_2 + 2KI = 2KCl + I_2.$$

The contents of the vessel, which have turned yellow or

brown, are poured into a beaker, rinsed twice with water, and the iodine is then determined by titration with decinormal sodium hyposulphite with the addition of starch. (See *Sulphurous Acid.*)

$$2Na_2S_2O_3 + I_2 = Na_2S_4O_6 + 2NaI.$$

One cubic centimetre decinormal sodium hyposulphite (24·8 *grm.* per litre) takes up 12·65 *mgrm.* iodine, and consequently corresponds to 7·98 *mgrm.* bromine or 3·54 *mgrm.* chlorine. As the air of factories is poor in these gases it is better to titrate with $\frac{1}{50}$th normal or centinormal hyposulphite.

Iodine Vapour.—We titrate here exactly as for Cl and Br; but it is not necessary first to liberate the iodine in the potassium iodide solution, as it is at once absorbed by IK.

Ammonia.—Absorption in 20 *cc.* decinormal sulphuric acid. The sulphuric acid is then titrated with baryta-water or dilute soda-lye, calculating the proportion of ammonia from the decrease in strength. Indicators are litmus or rosolic acid.

If we titrate the sulphuric acid with decinormal soda-lye, and have before the experiment consumed 20 *cc.* for saturating 20 *cc.*, but after the experiment only 17 *cc.*, the quantity of ammonia equivalent to $20 - 17 = 3$ *cc.* decinormal soda-lye, which has been absorbed, is $3 . 1·7 = 8·5$ *mgrm.* NH_3.

§ 136. Air is tested for *mercury vapour* (Renk, *Arb. aus dem Gesundheitsamt*, v.) by aspirating it slowly through a glass tube (20 litres per hour), fitted with a plug of wadding so as to keep back dust, and then through three to four light flasks arranged in a series and filled with leaf-gold. The flasks must have been previously dried and weighed together. After the completion of the experiment, air, which has been dried with sulphuric acid, is passed through, and they are weighed again. See also Von Raumer in the *Bericht über die zehnt. Versamm. der Bayrisch Chemiker*, 1891, Augsburg.

The qualitative, very sensitive demonstration (an inexperienced operator may possibly not succeed with the quantitative determination, as 1 *cbm.* of air contains generally

only 1 to 2 *mgrm.* mercury) is effected as follows: The leaf-gold from the flasks just mentioned is heated in a perfectly clean test-tube held in a horizontal position; or we may heat bright slips of sheet copper which have been suspended for some time in the air under examination. In the cold part of the tube there is formed a grey coating of metallic mercury, which takes a red colour (HgI_2 = mercuric iodide) if we throw in a very small particle of solid iodine whilst the tube is still hot. As organic matter interferes with the reaction, dust which has to be examined for mercury (the wadding from the above experiment) is placed in nitric acid, and the mercury so dissolved is precipitated upon a slip of bright copper which is held in the liquid. The iodine test can then be applied to the deposit thus obtained.

The methods for ascertaining in air the presence of aniline, nitrobenzol, and other compounds which react slowly are not yet sufficiently developed to find mention here.

III. *EXAMINATION OF THE AIR FOR SUSPENDED SOLIDS.*

1. Examination of the Quantity and Kind of Suspended Inanimate Constituents (Dust).[1]

§ 137. **Qualitative Demonstration of Dust according to Aitkin.**—If we allow a pencil of light to fall into a darkened room, the coarser particles of dust are distinctly visible as motes in the sunbeams. The minutest particles are made visible by enveloping them in a coating of water. A capacious flask is filled with water, which is then poured out in the room, save about 20 *cc.* The flask thus filled with the air in question is closed by means of a perforated caoutchouc stopper, through which passes a bent tube, open at both ends, and cut off short below the stopper. To the outer end is fixed a piece of flexible tubing, which is taken in the

[1] Quite recently Michaelis has constructed an ingenious apparatus for testing dust-respirators, for which we must refer to *Zeit. f. Hygiene*, ix. It determines the power of arresting dust, the degree of obstruction to respiration, &c.

mouth. If we suck at this tube, the air within the flask, which is saturated with moisture, is rarefied. Rarefied air can contain less watery vapour, so that a part of it is condensed, and deposited upon the particles of dust, which are thus rendered larger, and become visible. The experiment is most successful in a dark room, if we let a ray of light fall upon the flask. There is then formed, even with a moderate proportion of light, a dense iridescent cloud in the flask.

If we entirely empty a flask filled with water by means of a syphon, and cause the inflowing air to pass through a filter of wadding, the air is now free from dust, and the formation of a cloud does not take place.

If dust has to be microscopically examined, it is obtained by exposing in the room or space in question thin covering-glasses coated with glycerine. We easily recognise the characteristic forms of the fragments of textile fibres (see figure in § 416), the black, sharp-edged fragments of coal, transparent or translucent inorganic fragments of calcium carbonate, various plants, especially moulds and cells of woody fibre, &c. For systematic study a collection of material for comparison is necessary. For figures see Parkes, *Practical Hygiene*, Plates I. and II., 1891.

Quantitative Determination.—A larger quantity of air is aspirated through dense filters, which keep back all suspended matter, and which are constructed of layers of cotton or glass wool several centimetres in thickness. The plugs are inserted into a glass tube of 1 to 1½ *cm.* in width and of 5 *cm.* in length, to which is fixed a short, narrower tube. Before and after use the filters are dried at 100° for some hours, until the weight is constant. The increase of weight shows the quantity of dust in the air which has been aspirated. Ordinary aspirators will prove sufficient only if the air is very rich in dust. In general, therefore, we use water-air pumps and similar apparatus, which permit large quantities of air to be aspirated (*e.g.*, ½ *cbm.*). The volume of air is in this case to be measured by a gas-meter, and hence attention must be given to the rarefaction of the air which is measured with the manometer.

If it is required to separate organic and inorganic dust, the cotton plug is burnt to ashes after the second weighing, and the ash is determined. If from the figure obtained we deduct the weight of ash of a similar clean cotton filter, of equal weight when dry, we know the weight of the inorganic components of the dust. In many cases the question is to determine in the dust given metals (iron, lead, copper, &c.). We then dissolve the ash in dilute nitric acid, and proceed according to the general rules of quantitative analysis. See §§ 465 and 481.

2. Examination of the Air for Micro-organisms.

§ 138. If it is only required to demonstrate the quantity of germs in an air, and perhaps to obtain an indication of the degree of the presence of organisms, as well as of the kinds present, it is sufficient to expose flat capsules or plates with sterilised nutrient gelatine, to cover them again after a fixed time of exposure (two to thirty minutes), and to allow the individuals to grow up to cultures. Schizomycetes seem generally to float in the air, dried up on fragmentary substrata; the spores of hyphomycetes, on the contrary, are mostly free. According to Petri there are deposited upon a gelatine plate of 100 *scm.* in three to five minutes about as many schizomycetes as are contained in 10 litres of air. The spores of hyphomycetes attach themselves with difficulty, and very irregularly in separate experiments.

Quantitative investigations are often made according to the method developed by Petri. He filters the micro-organisms out of a current of air by means of fine, ignited quartz sand (particles of from $\frac{1}{4}$ to $\frac{1}{2}$ *mm.* in size). He passes the current of air through a tube fitted up as in Fig. 71. The tube, about 9 *cm.* in length and 1·6 *cm.* in width, is fitted in series with two sand-filters, each of 3 *cm.* in thickness. In order to charge the filters there are placed in the middle of the tube supports of the finest wire gauze; the filter S_1 is then filled, the support a_1 inserted, and the sterile wadding plug *w*; S_2 is then filled, and a_2 and a plug of wadding are

added, and the entire apparatus is then sterilised for one hour at 140.° When the tube is to be used the second plug of wadding is removed, the stopper *G* put in its place; *w* is taken out, and the tube is so arranged that a_1 points upwards, and a rapid current of air (10 litres per minute), measured by means of a gasometer (50 to 200 litres), is drawn through either by means of a water-air pump or a special pump devised by Petri. The difficulty of measuring this volume

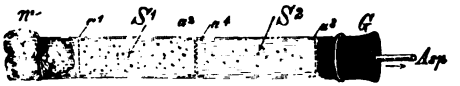


FIG. 71.—Petri's Tube.

of air is not trifling on account of the resistance of the sand-filter. Between the filter and the gas-meter there is inserted a manometer which enables us to read off at what rarefaction the quantity of air has been measured. If we are content with small quantities (10 to 30 litres), it is sufficient to attach to the tube an accurately gauged air-pump, and to draw through slowly by hand the required number of barrel-fuls of air, so that the atmospheric pressure has after each stroke time to recover its normal value within the barrel.

When the suction is completed the sand-filter S_1 is distributed into several culture capsules, into which liquefied gelatine is poured and well mixed up. The check-filter S_2 is treated in a similar manner, but it should not give rise to any culture. For particulars on this method and its history see Petri, *Zeit. f. Hygiene*, iii.

Various modifications have been proposed, such as filters of glass-wool, glass beads, sugar powder, &c., but they seem to be of no importance.

§ 139. The method of Hesse, which recently was accepted as the best, is useful, and often preferable in practice, notwithstanding its seeming complexity, as the method of Petri requires apparatus which is not everywhere available. A tube of 70 *cm.* in length and 3 to 4 *cm.* in width is fitted on the one side with a thick, perforated caoutchouc

stopper, through which is passed a small glass tube 1 *cm.* in width and 10 *cm.* in length, closed at each end with a compact plug of wadding. Over the other opening of the wide tube is drawn a caoutchouc cap (*a*) with a central aperture (*x*), and over it a second non-perforated cap. After the apparatus has been sterilised for one hour in the steam-pot the caoutchouc cap is eased, 50 *cc.* of sterilised gelatine are poured in, the stopper is rapidly closed, and amidst a gentle stream from the water-tap the congealing gelatine is distributed on the sides by a rotatory movement as in an Esmarch rolling-plate. If the tube is finally fixed horizontally in a stand there collects at the bottom a thick layer of gelatine not quite congealed. There is now attached to the smaller glass tubes an aspirator, the external caoutchouc cap is eased, and the air is allowed to stream through the tube at a speed of 1 litre in two minutes (by no means more rapidly) by opening the clamp of the aspirator. In consequence of the action of gravitation all the micro-organisms are deposited upon the gelatine plate, principally at the front part of the tube. The finest dust which can be demonstrated by Aitkin's method (§ 137) certainly remains suspended, but in successful experiments the filtering cotton plug *a*, distributed in gelatine and poured out on a plate, yields no cultures.

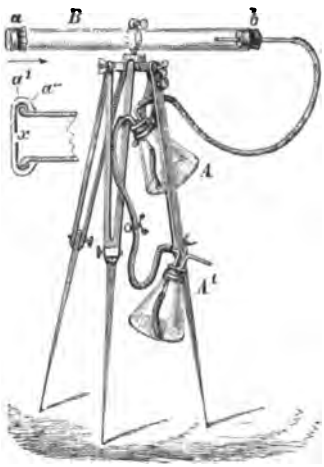


FIG. 72.—Hesse's Apparatus.

A distinction attaches to the results of both methods: in Hesse's method any groups of bacteria remain united, and form only a single colony; in Petri's method the groups are in part at least broken up, and thus show a higher number of microphytes.

Attempts have been made to carry the current of air

directly through large test-glasses full of liquid gelatine, and so to retain the fungi. If the entrance aperture, according to Strauss and Würtz, is made narrow, the temperature is kept at 26°, and frothing is prevented by a drop of oil laid upon the surface of the gelatine, the method is good if care is taken that the microbia do not find time to multiply. When the experiment is at an end the gelatine is rolled out, according to Esmarch, or capsule plates are cast. The apparatus can be worked with an ordinary aspirator; the best modification is that of Strauss and Würtz (*Ann. de l'Institut Pasteur*, 1888, and *Centralblatt f. Bakteriologie*, iv.).

IV. *GRAPHIC REPRESENTATION OF SERIES OF OBSERVATIONS.*

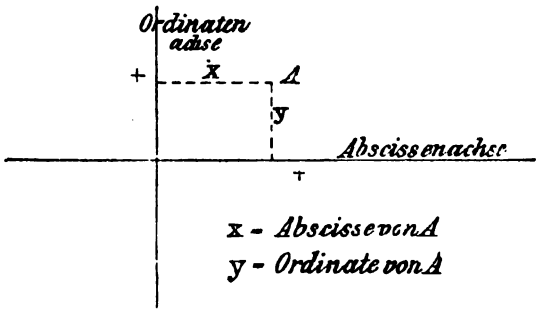
Appendix to Examination of Air.

§ 140. We very often obtain from our observations numerical results, which are not easily overlooked, but in which we can recognise regularities by graphic representation. As especially meteorological investigations (in close connection with which follow epidemiological observations) give occasion for graphic representation, some remarks on this subject may here be introduced.

In such representations we generally use an orthogonal system of co-ordinates; the abscissæ express the time, and the ordinates the numerical phenomena observed in this time (temperature, atmospheric pressure, &c.). Negative values are represented by ordinates drawn downwards, positive values by ordinates drawn upwards; negative abscissæ (which are scarcely used in hygiene) are carried out to the left. The only art in plotting out curves lies in selecting the right scale. If, *e.g.*, the question concerns a curve of temperature it is naturally in itself indifferent whether we take for one hour, one day or one year, 1 *cm.* or 1 *mm.*; and in like manner whether a length of 1 *cm.*, 1 *mm.*, or any other desired magnitude is taken as the expression for 1°, 0·1°, or 10°. The size of the scale is determined by the size of the sheet of paper; secondly, the number of observations; and

thirdly, the maxima and minima which have to be entered. It is, of course, not always desirable to make the sum of the abscissæ on the one hand, and on the other the largest ordinate, as large as it is possible to do on the paper in use. It is generally preferable to obtain curves of a medium course. If the abscissæ are selected too long the curves are too extended, and in the opposite case, *i.e.*, if they are too short and the ordinates too long we obtain too sudden curves.

We often discover whilst drawing that the scale must be altered. An absolutely arbitrary choice is certainly limited



EXPLANATION OF TERMS.
Ordinatenachse = Axis of ordinates. *Abscissenachse* = Axis of abscissæ.
Abscisse von A = Abscissa of A. *Ordinate von A* = Ordinate of A.

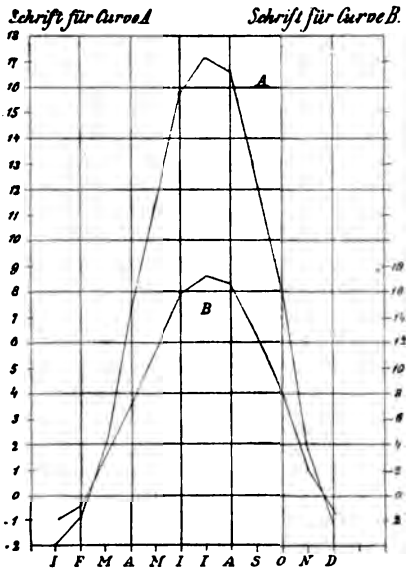
FIG. 73.—Orthogonal Co-ordinates.

by convenience. In general we use for drawing curves so-called millimetre paper—a paper marked in squares by means of fine lines at the respective distances of 1 *mm.*, with thicker lines at 5 *mm.*, and with thicker again at 10 *mm.* On this account we take a unit of measurement, be it a metre, a degree of temperature, a day or an hour, as 1, 5, or 10 *mm.*, whereby the measurement in plotting out the curves is simply converted into counting the squares. If none of these systems of lines is suitable, and if we must, *e.g.*, take 2 or 3 *mm.* as unit, it is convenient to draw every second or third line with pencil in order to plot out the curve more rapidly.

This is best shown by examples: 1. The progress of the mean annual temperature at Bayreuth is to be represented

from twenty-seven observations of monthly averages. The mean values obtained are (Renk, *Die Luft.*, p. 22).

January . . .	−1·9	July	17·2
February . . .	−0·9	August . . .	16·6
March . . .	2·3	September . .	12·8
April	7·5	October . . .	8·1
May	11·6	November . .	1·9
June	15·8	December . . .	−1·4



EXPLANATION OF TERMS.
Schrift für Curve A = Lettering for Curve A. *Schrift für Curve B* = Lettering for Curve B.

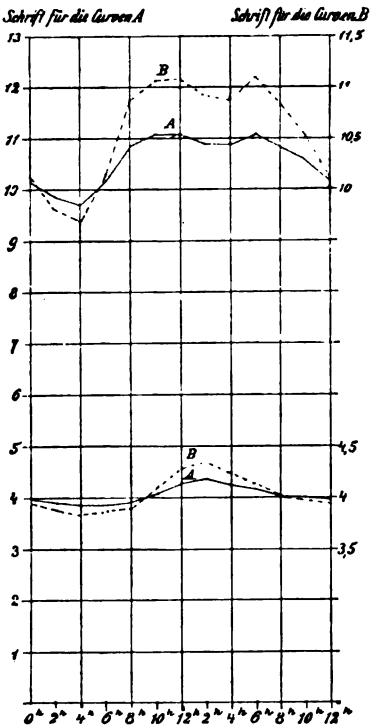
FIG. 74.—Progress of the Mean Annual Temperature at Bayreuth.

As 1 *cm.* per month would give a curve of 12 *cm.*, too long for our space, we take accordingly ½ *cm.* per month. For 1°, 1 *cm.* is too long, but ½ *cm.* is possible; the curve *A* is drawn on this assumption, but still more suitable is 1° = ¼ *cm.*, on which assumption we obtain the curve *B*. The latter is plotted out so that the temperature is divided by 2, and the curve then constructed on the assumption that 1° = ½ *cm.*, millimetre paper being used.

2. The progress of the mean temperature in winter and summer at Munich is to be graphically represented according to 2-hourly observations.

The numbers given are :—

	Winter.	Summer.		Winter.	Summer.
Midnight . .	3·96°	10·11°	Noon . .	4·27°	11·06°
2 A.M. . .	3·89	9·84	2 P.M. . .	4·33	10·90
4 „ . .	3·84	9·69	4 „ . .	4·22	10·86
6 „ . .	3·86	10·12	6 „ . .	4·12	11·08
8 „ . .	3·89	10·85	8 „ . .	3·99	10·86
10 „ . .	4·06	11·05	10 „ . .	3·97	10·53



EXPLANATION OF TERMS.

Schrift für die Curven A = Lettering for the Curves A. *Schrift für die Curven B* = Letter ing for the Curves B.

FIG. 75.—Mean Daily Temperature at Munich, in Winter (lower curves) and Summer (upper curves).

½ *cm.* again is quite suitable. For 1° we assume the largest possible value, as the curves would otherwise be very flat. If we draw the two curves independently, we may use any scale at pleasure; *e.g.*, 1° = 2 *cm.* (the dotted curves), but it is more suitable to use the continuous lines, in which 1° is only = 1 *cm.*, but in which the respective distance of the two curves is drawn exactly in proportion to the curves themselves.

3. The monthly rainfall of the year 1885 at Würzburg is to be expressed graphically. As the quantities of rain are distributed over an entire extent of the abscissæ, they are preferentially expressed by a plane erected upon the abscissa. In Fig. 78*a* equilateral triangles have been selected, but rectangular figures might have been used in their stead. But we might also be satisfied with a line (Fig. 78*b*), erected in the middle of the portion of the abscis which meets the month.

January . . .	13·6	August . . .	48·8
February . . .	48·0	September . . .	55·9
March . . .	50·0	October . . .	68·4
April	4·2	November . . .	5·3
May	94·4	December . . .	56·0
June	18·9		
July	69·6	Total . .	586·1

If it is required to exhibit the mean values of several days, several years, &c., double curves are especially valuable for showing periodicity. We simply connect together the annual curves obtained in pairs, and can thus follow graphically the transit from December to January.

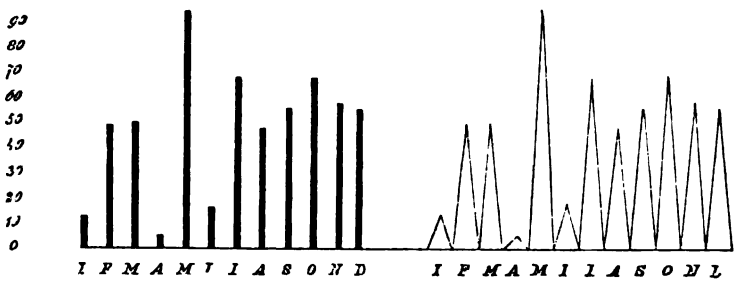


FIG. 76*b*. FIG. 76*a*.

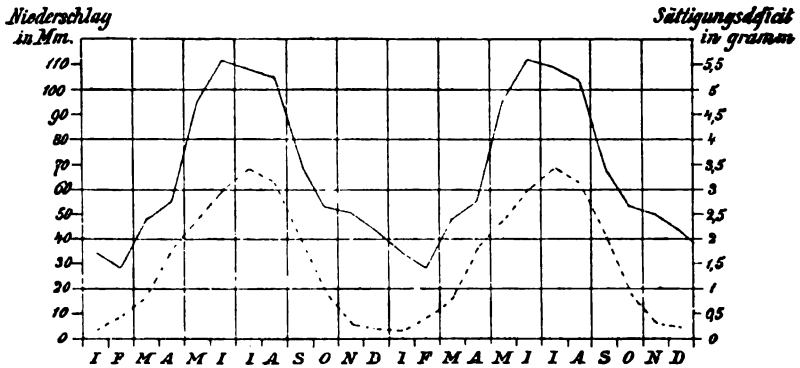
Quantities of Rain at Würzburg in the Year 1885.

We often have to exhibit upon one and the same sheet two quite distinct occurrences, in order to be able to follow the dependence of both on time, and possibly on each other. Then of course the same extent of ordinates must show, *e.g.*, simultaneously, *a*, the downfall in millimetres, and *b*, the deficiency of saturation in grammes.

Fig. 77 gives for the average of twenty-eight years the quantities of downfall and the deficiency of saturation at Munich. The double curve displays the following numbers (1 *cm.* ordinate representing at once 40 *mm.* of downfall and 2 *grm.* deficiency of saturation):—

Munich, Average of Years 1856–1883 (28 *Years*).

	Jan.	Feb.	March.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
Mean downfall, in millimetres	35·5	29·6	48·5	55·6	95·1	111·9	108·8	104·4	68·1	53·1	50·0	42·9
Mean deficiency of saturation, in grammes	0·15	0·41	0·81	1·78	2·34	3·00	3·43	3·13	1·98	0·93	0·30	0·20



EXPLANATION OF TERMS.
Niederschlag in Mm. = Downfall in millimetres. *Sättigungsdefizit in gramm.* = Deficiency of saturation in grammes.

FIG. 77.—Double Annual Curve of Downfall (——) and of Deficient Saturation (·····) at Munich.

§ 140*a*. Closed curves obtained by the use of polar co-ordinates display very finely the cyclical course of regular natural phenomena. The ordinates are here plotted out on radii proceeding from a centre; the angles between the radii express the intervals of time. If the time to be thus represented extends to a year, we have for each month an angle of $\frac{360}{12} = 30°$; if a day only is to be exhibited, $\frac{360}{24} = 15°$ is the angle corresponding to an hour. In this method, in which the scale for the ordinates is of course optional, the curve (Fig. 77), with an equal length of ordinates, has the following appearance (Fig. 78). This method is not practical if great fluctuations occur in the single radii (months); the choice of a scale is then difficult, since the maximum must find room upon the paper in two different directions. But if the maximum is selected small, the minimum is very small, and the curve becomes indistinct.

The polar co-ordinates have a particular signification in the representation of so-called wind-roses. Very different wind-

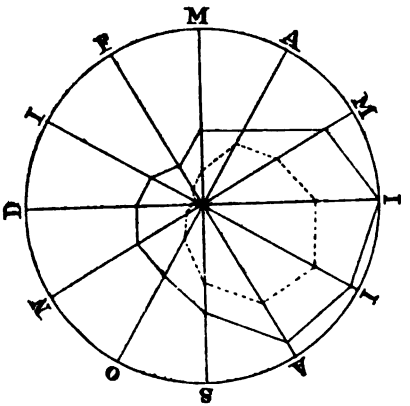


FIG. 78.—Fig. 77 plotted out on Polar Co-ordinates.

roses may be constructed. The radii run in the main directions of the wind-rose; their length refers to any natural phenomenon which occurred during the direction of the wind in question, according to the frequency or strength of the phenomenon referred to. Thus, by a *baric* wind-rose we understand a drawing in which there is plotted out upon each radius of the wind-rose a value proportional to the

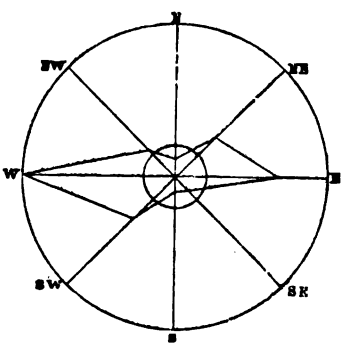


FIG. 79.—Anemographic Wind-rose for Munich.

mean annual position of the barometer in this direction. A baric wind-rose shows at a glance the higher atmospheric

pressure during north and east winds, as compared with the south-west wind. In an umbric (pluvial) wind-rose the length of the radii corresponds to the mean quantities of downfall in the corresponding direction of the wind for an average of years. Wind-roses might also be constructed showing the connection of the direction of the wind with the number of rainy days. In like manner there are thermic, nephic (cloudiness), &c., wind-roses.

Anemographic wind-roses may also be constructed of two kinds: on the one hand the mean length of the radii shows the mean intensity (dynamo-anemographic wind-roses), or on the other the mean frequency (ordinary anemographic wind-rose).

A wind-rose of this latter kind is shown as an example.

Fig. 79 expresses the mean observations made during all the thirty-eight years (1843 to 1880) at Munich.

N.W.	3·28
N.E.	11·42
E.	21·08
S.E.	4·08
S.	1·78
S.W.	11·20
W.	33·09
N.W.	7·64
Calm	6·54, expressed by the small inner circle of the figure.

B. Conclusions as to the Air.

1. Air in the Open.

§ 141. In our climates the air has rarely any demonstrable properties directly injurious to the health. As the nature of the free air withdraws itself from our influence, the materials for its hygienic examination are very scanty. Further, individual constitution and habit have a great influence on the question of the effects of any peculiar character of the air. It is also very difficult to consider the air in its influence upon health completely isolated from other agencies. We may say in general that in a calm, a temperature from $-15°$ to $+25°$ can be borne without inconvenience by a healthy man,

suitably clothed, and not working hard; in strong winds, $-5°$ and $+30°$ may be taken as the limits. A high moisture (slight deficiency in saturation) may render motionless air very unpleasant from $+20°$ upwards: especially if muscular movement is exerted, and if the clothing interferes with the escape of heat (soldiers on the march), the danger of sunstroke (illness and death from overheating the body) sets in from about $+20°$ and upwards. Inversely, a very high deficiency of saturation and violent motion renders the air oppressively drying, even at relatively low temperatures (20° to 25°). Temporarily a man may bear much higher and lower temperatures without injury, $-40°$ to $+100°$, or even 110°; for the endurance of high temperatures it is especially important that the air must be dry, and that the changes of air are not too abrupt, especially if the body is covered with sweat.

§ 142. If a decision about a climate is to be drawn from observations of temperature collected for many years, the following points must be taken into consideration, and the following values to be calculated:—

1. The mean temperature of each month in the course of years. We can thus judge if the climate has hot summers and cold winters (a continental climate), or a more uniform temperature throughout the year (sea or insular climate). Every climate which has for continuous weeks temperatures decidedly below $-10°$ or above 25° is at such times found unpleasant by unaccustomed persons, and requires especial adaptation in dwellings, in clothing, and in the entire manner of life.

A climate may be briefly characterised by the mean annual fluctuation, *i.e.*, the difference between the temperature of the hottest and the coldest month.

A climate with a mean yearly fluctuation of at most 15° is considered by Supan as an equatorial or oceanic climate; from 15° to 20° a transition climate, from 20° to 40° as a land climate, and above 40° as an extreme land climate. A further point for judging of the temperatures observed appears as follows: according to Supan the warm zone extends from the equator on each side to the annual isotherm of 20° (a

line connecting all points in which the yearly mean amounts to 20°). The temperate zone extends from the isotherm 20° to the isotherm 0°, beyond lies the frigid zone. The torrid zone is divided into a tropical and ektropical belt by a line connecting all points in which the temperature of the coldest month does not fall below 20°. In like manner the temperate zone is divided into an equatorial and a polar belt by a lined rawn through all points, in which the coldest month does not fall below 0°. Germany lies in the polar belt of the temperate zone.

The mean annual temperature (obtained on dividing by twelve the sum of the monthly averages) has very little value. The same holds good of the daily curve in the average of the year, as by this form of statement almost everything of hygienic importance is eliminated.

2. A knowledge of the mean monthly temperatures is far from being sufficient for a hygienic decision. A second important point is the knowledge of the movement of temperature of an average day (of the daily period) in each month. For this purpose the morning, noon, &c., temperatures for each month are added up separately and divided by thirty (thirty-one or twenty-eight). The more years of observation are available for preparing curves of these mean values the more accurate is the result.

The difference of the temperature of the hottest and coldest hour of the day in such an average day is called the periodic daily fluctuation.

The hygienist is even more concerned about the aperiodic than the periodic daily fluctuation of temperature, *i.e.*, the difference of the means of all the maximum and all the minimum temperatures in each month in a series of years. The aperiodic fluctuation, of course, exceeds the periodic.

Generally, if the monthly means are not too high or too low, a slight fluctuation of the temperatures at different hours of the day is found the most suitable (oceanic-climate as compared with land-climate). If, however, the mean temperature is very high, in our parts a greater daily fluctuation, *i.e.*, a hot noon-day and a cool night (*e.g.*, by the influence of mountains) is generally more pleasant than a continuous temperature of mean height. The house protects us against the heat of the day, and the coolness by night favours sleep. But if the daily fluctuation exceeds 15° or even 20° it becomes unpleasant, *e.g.*, day temperature, 28°; night temperature, 8°. We experience this, especially

when travelling, if protection by clothing and shelter is not available in the customary manner.

In very cold climates a day temperature, raised by solar radiation, though the nights are frosty, has advantages for man as compared with a uniformly low temperature (winter on the coasts) if only suitable dwellings are available.

3. But even the knowledge of the average daily movements of temperature (daily periodic amplitude) in each month does not suffice; we must further know whether—what the published mean values never reveal, and what may better be ascertained from the original observations—abrupt changes of temperature in the course of a day, or of a few days, are frequent or indeed customary. The decisive points for the formation of an opinion are:—

1. *The Mean Maxima and Minima of each Month in the Course of Years.*—The highest temperatures shown by each month in the course of years are added up, and the sum is divided by the number of years. In this manner we obtain the mean maximum for the month in question. The mean minimum is found in the same manner. The difference gives the mean (aperiodic) amplitude of the monthly fluctuation.
2. *The Mean Difference between One Day and Another in each Month.*—To this end we add the differences of temperature of every two successive days through the entire month (whether the day is warmer or colder than the foregoing its sign is always positive), and divide by the number of days. The more years can be employed the better.

For deciding on the character of the climate of each month a knowledge of the mean absolute maximum and minimum temperatures of each month and each year is of interest. Lastly, we may mention the maxima and minima hitherto observed for each month in the course of years.

As an instance we may here mention the values for Vienna for the hottest and coldest month, as discussed by Hann. Mean yearly fluctuation = 22·2.

Month.	Monthly or Yearly Mean for 100 Years.	Single Monthly or Yearly Means Differ from Average by a Mean.	Mean Temperature.			Daily Fluctuation.		Mean Absolute Monthly and Yearly Extremes.		Difference of these Extremes.	Absolute Extremes Observed.		Average Change of Daily Temperature.
			6 A.M.	2 P.M.	10 P.M.	1. Periodic.	2. Non-periodic.						
Jan.	− 1·7	2·3	−2·3	0·3	−1·6	2·7	4·9	9·7	−12·1	21·8	18·8	−25·5	2·1
July	+20·5	1·3	16·9	24·3	18·9	7·9	10·1	32·6	11·0	21·6	38·8	8·0	1·9
Year	+ 9·7	0·74	7·1	12·9	8·8	5·9	8·0	33·9	−15·1	49·0	38·8	−25·5	1·9

If briefly summed up the extremes of the absolute temperatures of a locality have most interest for the hygienist. Otherwise it is most important to know how suddenly the fluctuations of temperatures take place in short intervals. Considering the great adaptability of the healthy human system these indications are sufficient, and in other respects we must refer to the text-books of climatology and climatotherapy.

§ 143. For determining radiant heat numerical statements are hitherto wanting; every one knows how much the difficulty of bearing high atmospheric temperatures is intensified by radiant heat, and that of low temperatures facilitated. The action of solar radiation is especially intense in the Alps, where the rarified atmosphere, poor in watery vapour, does not absorb them nearly to such a degree as in the lower lands. The beneficial action of the sun's rays is shown in the abundant Alpine flora (as compared with that of the polar regions); in the possibility of sitting in the open air in the winter health resorts, though the temperatures are below 0°; the injurious action of a too intense radiation is seen, *e.g.*, from the scorching of the face when wandering among the glaciers.

E. Frankland observed with the vacuum thermometer, the height of the sun being alike :—

Shade.	*Sun.*	*Place.*	*Altitude.*
30·0°	41·5°	Oakland Park	46 *m.*
32·2	37·8	Whitby	20 „
26·3	44·0	Pontresina	1800 „
14·2	47·0	Gornergrat	3140 „
6·0	59·5	Diavolezza	2980 „

The indirect radiation from rocks previously heated by the sun, and the direct reflection from rocks (two thermometers with blackened

bulbs showed differences up to 10°) and sheets of water, is very important for men and for the cultivation of the vine.

Rubner (*Lehrbuch d. Hyg.*, p. 71) has ascertained that the solar radiation in Germany is sufficient (supposing the heat to be entirely absorbed) to cause 0·7 to 0·4 calories to be absorbed by perpendicular surfaces of skin per minute and per square centimetre. This is very much, if we consider that the naked body at the ordinary temperature of a dwelling-room radiates out only 0·14 calories per square centimetre of skin. He established also by a thermo-electric process that the skin of the face is sensitive to radiant heat if 0·036 calories per minute fall from a gas-burner upon 1 *scm.* of skin; 0·059 calories is troublesome, and 0·115 calories annoying to a high degree.

The strong nocturnal refrigeration on mountains by irradiation is hygienically indifferent, as the houses afford protection; but, of course, serious to persons passing the night in the open.

§ 144. The slight daily regular fluctuations of the barometer (maximum in the early morning, and minimum at mid-day) are without any influence upon our condition, and even the great irregular changes, *e.g.*, 20 *mm.* in twenty-four hours, have no influence upon a healthy man. An explanation of the state (often typical hemicrania) which many persons experience when the barometer is low, *e.g.*, regularly before and at the beginning of the winds, known in some parts as Fœhn, elsewhere as Scirocco or Solano, has not as yet been found. The low state of the barometer cannot be the only cause, since the same persons feel well on mountains where the barometer falls much lower.

The decrease of atmospheric pressure upon high mountains becomes unpleasant to the majority of persons not acclimatised at about 2000 *m.* (in some cases even from 1000 to 1500). Dryness of the mucous membranes (from the increased exhalation of watery vapour), accelerated respiration and pulsation, sleeplessness and excitement often annoy new-comers, but they generally become quickly acclimatised. Serious morbid phenomena, owing to the decreased tension of oxygen (mountain-sickness), generally make their appearance only at 4000 *m.* (height of the barometer, 460 *mm.*). The air behaves like one containing only 12·6 per cent. of oxygen does at a pressure of 760 *mm.* Increased transformation of matter by cold or exertion, which cause an increased demand

for oxygen, promotes illness. But different relations also occur; acclimatisation is effected by the increased strength of the respiratory muscles, and, it is alleged, by the augmentation of the hæmoglobine—especially in countries which possess sufficient heat at these altitudes, in virtue of their equatorial situation. We cannot here discuss the action of an extremely reduced atmospheric pressure (balloon ascents) nor that of its considerable increase (engineering operations in compressed air). See Renk, *Die Luft;* or Paul Bert, *La Pression Barometrique.*

The height of the barometer (or its fluctuations) are, as it is well known, much observed for the sake of an indirect forecast of rain. The fact that with us warm, moist west and south-west winds, which as a rule bring rain, generally blow at the time of a low atmospheric pressure, has passed to such an extent into the popular consciousness that the approach of rain is universally inferred from a fall of the barometer—often wrongly. A much more certain prognostication may be based upon the simultaneous observation of the atmospheric moisture and the temperature of the air. If the air is warm and moist, rain is probable on any decided fall of temperature; if the air is cold, the arrival of a current of warm, moist air will probably bring rain. Weather-charts with graphic representations of the isobars (lines of equal atmospheric pressure, *i.e.*, barometric observations reduced to the sea-level), and the comparison of these representations day by day, are important auxiliaries for weather forecasts. We know that places towards which the barometric minima move or advance have mostly to expect rain, and this the more certainly and rapidly the steeper the declivities of the atmospheric pressure—or, in other words, the closer the isobars follow each other.

In the maximum regions there prevails mostly dry, clear weather of a settled character; these regions change their position but slowly, whilst the minimum regions are rapidly displaced, leaving, in the northern hemisphere, the maximum region generally on the right. This ensues because the atmospheric pressure does not become equalised at right

angles to the isobars, but the wind is deflected, owing to the rotation of the earth. The wind blows in such a manner that, if we have it at our back, the maximum of the barometer does not lie exactly behind us, but to the right and behind, whilst the minimum lies in front and to the left.

It is, of course, not our province to enter upon the difficult art of weather prediction. Great experience and a close attention to local conditions are necessary, as well as a regard to the above rules. See Günther, *Meteorologie*.

§ 145. The proportion of watery vapour in the air has as much influence upon our bodily condition as the temperature and the barometric pressure. Flügge first showed, and more recently his pupil Deneke (*Zeitschrift f. Hygiene*, i.), has fully demonstrated that the deficient saturation of the air is the value which shows most clearly the hygienic signification of the proportion of water in the air. The more water an air can still take up, the more water it withdraws from the body: the deficiency of saturation can continually reach higher values as the temperature rises, and is very great only at high temperatures.

A great deficiency in saturation may act upon us in two ways:—

1. The withdrawal of water from the skin is increased; we experience thirst, and a reduced secretion of urine.
2. The mucous membranes accessible to the air (nose, fauces, lips) are desiccated; they may crack, give pain, and afford entrance for pathogenous matter. The desiccation of the fauces increases thirst locally, and renders it painful.

In cold air the deficiency of saturation is always very small (at 0° it cannot exceed 4·9 *grm.*, at -20^{c} it does not exceed 1·1 *grm.*), so that we might infer cold air could not withdraw much moisture. But if we reflect that the cold air, by becoming heated in our respiratory organs to about 30°, becomes an air with a very great deficiency of saturation (up to 30 *grms.*), and reflect that the air expired is saturated with watery vapour, we comprehend the thirst of Polar travellers.

The heated air of the clothing has also a very great deficiency of saturation. For the moisture thrown off by man, it is tolerably indifferent whether in very cold air the deficiency of saturation is somewhat greater or smaller; it cannot fluctuate much.

At mean temperatures nothing is known with certainty as to the upper limit of the deficiency of saturation which we feel so pleasant in the open air; probably we are not very sensitive. At Rostock the average deficiency in winter is 0·4 to 0·5; in summer, 4·7 to 5·6 (Uffelmann).

At high summer temperatures, high deficiencies of saturation may occur; at 8 to 9 *grm.* we experience, according to Deneke, a certain oppression, whilst deficiencies of 16 to 20 *grm.*, such as are not rare with us in hot summers, cause the air to feel intolerably dry.

Air which is too moist may prove much more dangerous than such as is too dry. At low temperatures, as already said, an air even saturated with moisture may excite thirst. Certainly a cold, foggy air is especially unpleasant, though not so much on account of its moisture as of the various matters which we inhale along with the mist-globules. Further, air rich in moisture, which saturates our garments, facilitates the loss of heat, which at low temperatures, and to persons thinly clad, is felt as very trying. At mean temperatures (15°), if suitably clothed, and not working too hard, we feel quite comfortable in an atmosphere saturated with watery vapour, *e.g.*, in rainy weather. But from the moment when the temperature of the air rises so that a secretion of sweat (that is, refrigeration by the rapid evaporation of sweat) appears necessary to preserve our normal temperature, a slight deficiency of saturation checks at once the evaporation of the sweat, and consequently arrests the cooling process. The temperature of the body then rises, especially if work and unsuitable clothing co-operate, and the danger of sunstroke (heat-apoplexy) is at hand.

How far the deficiency of saturation must descend in order to produce dangerous conditions at temperatures of 20°, 25°, 30°, we have hitherto no numerical determinations.

From the considerations aforesaid it is plain that the deficiency of saturation in itself is far from sufficient to explain the influence of the air upon our health. It is felt very differently according to the prevailing temperature. As the temperature rises, the margin of the deficient saturation which we feel pleasant becomes considerably narrower.

The velocity of the wind has also a very considerable influence—though hitherto not capable of numerical expression—upon our subjective estimation of the atmospheric moisture, at least at high temperatures. The more air sweeps over our bodies the more easily are slight deficiencies of saturation endured, but the less easily are great deficiencies, and this for tangible reasons. A sultry air is quite as often a quiescent, warm air with a considerable deficiency of saturation, as one which is in reality abnormally rich in watery vapour. Many observations are therefore still wanted in order to yield valid foundations for a hygienic conclusion on the proportion of watery vapour in an air.

Generally we shall give a decided preference to a climate with dry although hot air, rather than to one with a hot and damp air. Besides the extremely relaxing effect of moist heat, we must consider that damp, hot localities have generally to suffer in a signal manner from endemic diseases: malaria, cholera, dysentery, &c. See Reinhard, *Arch. f. Hygiene*, iii.

Since these considerations were penned, Rubner, by a series of highly important researches, has begun to place the entire question of the import of atmospheric moisture for man upon new foundations. The matter has shown itself to be essentially more complicated than was formerly assumed. The elimination of water is by no means a passive act in the degree supposed. It depends upon an entire sum of single conditions (relative dryness of the air, temperature, kind of diet, clothing), and therefore appears as one of the main conditions for the regulation of heat. The researches should be studied in the original. A copious abstract by the present author is found in the *Münch. Med.*

Wochenschrift, 1891, No. 1. A few practical points only can be brought forward here.

The need of giving off heat by any creature is the greater the less readily it can keep its bodily temperature constant in any other manner. Cooling in other ways is interfered with—

1. By too high a temperature of the ambient air, as thereby conduction and radiation are checked.
2. By warm clothing, and consequent disturbance of the outflow of heat.
3. By abundant food. In this respect hot-blooded animals are wanting in the important power of generating less heat by the restriction of metabolism, as only the production of muscular heat, but not that of glandular (?) heat, is to some extent dependent upon its will.

In all these cases a relatively moist air is therefore unpleasant. Rubner has shown that in relatively moist air the thermo-conductivity of the skin, and consequently the cooling power, decreases; but this is in most cases not sufficient entirely to overcome the rise of heat. In cold weather, on the other hand, a relatively high moisture can act unpleasantly by rendering the clothes and the naked skin better conductors.

Rubner further, according to his way of observation, arrives—as do I above in the text—at the conclusion that the amplitude of atmospheric dryness which is agreeable to us at high temperatures is smaller than at low temperatures. If at 20° an air of 70 to 40 per cent. relative dryness (30 to 60 per cent. relative moisture) is felt as admissible, this amplitude for 25° is narrowed to 65 to 38 per cent., and for 7° is enlarged to 95 to 55 per cent. At low temperatures no great fluctuations of the deficiency (perhaps 0 to 4) are possible, and they are unimportant; at high temperatures, where the deficiency may vary from 4 to 30 per cent., it may be very important that the air is neither too moist nor too dry. The hotter the air, the more important is its relative moisture. (Rubner, *Arch. f. Hygiene*, xi.).

§ 146. On the degree of cloudiness depends in part the supply of direct sunlight, the enormous importance of which for the various chemical and biological processes cannot yet be perfectly estimated. Some of its most important effects are: sunlight increases metabolism in man and other animals; in sunlight alone is carbonic acid assimilated and oxygen given off by green plants; sunlight kills bacteria and their spores; and lastly, it exerts a very considerable psychical influence (Raum, *Zeitschrift f. Hygiene*, vi.).

An account of the number of days with total clouding and with a clear sky, and further with days of cloudiness above and below 5, completes the picture of a climate. Still more important is a statement of the daily and monthly number of hours of sunshine, which may be registered photographically by exposing sensitive paper driven by clockwork past an aperture.

Thus, in Vienna, February has 100·8 and March 141·8 hours of sunshine; at Davos, February has 124 hours and March 186 hours.

§ 147. The observation of the downfall is, from various grounds, of hygienic interest; here we can merely give intimations.

1. The quantity of the downfall and its distribution (the number of rainy days) has a great influence upon the climate, and therefore upon man. Atmospheric moisture depends, in the first place, upon the downfall in connection with the temperature, and affects the entire character of vegetation.

Whilst Eastern Germany has a yearly downfall of only 300 to 400 *mm.*, from 500 to 600 *mm.* are often found in the plains of Central Germany. In the Central Mountains of Germany (Thuringerwald) the quantity rises to 900 to 1000 *mm.* The Harz, the Black Forest, and the Vosges have 1400 to 1500 *mm.*; in the Boehmerwald (mountains between Bohemia and Bavaria) and in the Alps 2000 to 2500 *mm.* have been observed. In Scotland and Wales there are localities with quantities up to 4182 *mm.*, and in the tropics quantities of rain from 5000 to 7000 *mm.* are very common. In India as much as 12,520 *mm.* has been observed.

It might prove difficult to show a direct influence upon

human health of the fluctuations, as observed in various localities in Germany; the indirect influence has not yet met with sufficient attention.

2. The downfall affects in the most striking manner the appearance and the course of infectious diseases (cholera, typhus, malaria, splenic fever): relations have been especially detected in the case of cholera. A careful observation of the quantity and the distribution of the downfall (and the level of the ground-water; see Water) has explained already many a perplexing rhythm in the course of epidemics, and other peculiarities—although it is not yet possible to pronounce with certainty why in most places slight rainfalls favour the occurrence of cholera-epidemics, whilst heavy rain hinders them. Von Pettenkofer interprets the observations so that a certain mean moisture of the soil plays a decisive part in the occurrence of epidemics. The course of the deficiencies in the saturation of the air shows surprising relations to the level of the ground-water (Soyka, *Der Boden*, p. 305).

§ 148. The great hygienic significance of the winds—direct and indirect—can merely be glanced at. We feel currents of air the more distinctly the smaller and wetter the part of the skin which they encounter. The larger a moist naked part of the skin which meets the wind, the more unpleasant to us does it generally become. A velocity of wind of 0·16 to 0·25 *m.* is felt only if small surfaces of the skin, quite naked, of 14 to 30 *scm.*, are struck by the wind. The entire clothed body perceives the motion of the air only at or above a velocity of ½ to 1 *m.* A mean average velocity of the wind of 2·6 *m.* is considered high; 1·6 to 1·8 *m.* are more frequent values, but it is very important to know how the intensity of the wind and its direction are distributed over single months and days.

As above mentioned, strong winds render dry air, cold or hot, less bearable, but they facilitate the endurance of moist, warm air. Winds which blow periodically, occasioned by the unequal heating of plains (or water) and mountains

(islands or coasts), have generally a beneficial influence upon human health in hot climates, or with us in summer (*e.g.*, Munich). On the other hand, warm valleys protected from the wind are suitable places of abode for certain invalids. Winds which raise up clouds of dust are the torment of many a city.[1] In Central Germany the highest temperatures are brought in winter by S.W. and W. winds, and in summer by S.E. winds. The lowest temperature accompanies N.E. winds in winter, and W.N.W. winds in summer.

Indirectly the winds may be of extreme hygienic importance, since dry warm winds desiccate the soil, whilst moist winds bring rain, and with the rain either an increased or a diminished disposition to infectious diseases, *e.g.*, the monsoons in India. Malaria seems capable of being diffused by winds to a distance (crews of ships become ill if they sail too closely along a coast haunted by fever).

§ 149. The proportion of carbon dioxide in the open air is constant in the country, 0·3 per thousand; in towns it amounts to 0·4 per thousand; in both cases it has no hygienic meaning.

As to the signification of the small traces of ozone,[2] H_2O_2, N_2O_3, NH_3.[3] &c., we know nothing positive. More important is occasionally the impregnation of the air with agreeable odours (fir-woods, aromatic plants in bloom), or evil odours, the action of which upon the mind is distinct, and must not be overlooked as regards the general health. Accurate investigations on these difficult questions are wanting. Habit plays here a very important part (see § 456).

Sulphurous acid and hydrochloric acid, even in very small traces, damage vegetation (especially conifers and gra-

[1] And also of country districts in Africa and Australia (dust-storms).—*Editor.*

[2] As the air of inhabited rooms is almost always found free from ozone, since it is assumed to be decomposed by the organic products liberated, hygienists must for the present regard air rich in ozone as pure.

[3] At Paris and Pesth, NH_3 is present to the extent of 0·022 to 0·033 per cubic metre. In other places alleged values have been found up to 5 *mgrm.* per cubic metre = 0·0065 per thousand.

mineæ),[1] also metallic objects, *e.g.*, telegraph wires, and they are probably not without influence on human health, even in the proportions in which they occur near manufactories; certainly they are often very unpleasant.

§ 150. A high proportion of dust in the open air is very annoying. Tissandier found in the air in the country, after rain, 0·25 *mgrm.*; in dry weather, 3 to 4·5 *mgrm.* dust per cubic metre. In Paris there was, after rain, 6 *mgrm.*; in dry weather, 23 *mgrm.* Fodor found at Pesth, as the average of many observations, 0·24 *mgrm.* in winter, and 0·55 *mgrm.* per cubic metre in summer, therefore very low values. Lime-dust has an especially bad reputation, as it is said to produce phthisis.

Bacteriological examinations of the air in the open have hitherto yielded few results of practical importance; the air on high mountains and over the open sea is entirely or approximately free from fungi. Pathogenic organisms have hitherto never been found in the open air; the number of micro-organisms in 1 *cbm.* of the open air fluctuates greatly, thus to furnish a point of comparison there have been found:—

		Bacteria.	Hyphomycetes.
Petri, Berlin.	Court of Hyg. Institute.	0–1071	215–810
„ „	Roof of Hyg. Institute.	330–510	1190–1240
Frankland and Hart.	Roof at South Kensington, London	400 (Jan.)–10,500 (Aug.)	
Miquel, Paris.	Summit of Pantheon	200	...
„ „	Park of Montsouris	480	...
„ „	Rue de Rivoli	3480	...
Uffelmann, Rostock.	Sea-shore	50–300	Of these ⅔ are schizomycetes, and ⅓ hyphomycetes.
„ „	Open field	150–750	
„ „	Court of University	150–1300	

The causes of these fluctuations are in part easily intelligible; the fluctuations themselves are for the present without practical interest.

[1] The walnut-tree is perhaps of all trees the most readily destroyed by acid fumes.—*Editor.*

2. The Air in Closed Dwelling-Rooms.

§ 151. In closed dwelling-rooms, where the air is more subject to our will, our requirements for comfort are more definite.

1. The temperature of the air must range between 15° and 20° Centigrade, according to the bodily or mental work taking place in them.[1] In theatres, music-halls, &c., the temperature is often allowed to rise to the oppressive height of 25°. The more general introduction of the electric light will, it is to be hoped, soon cause such a temperature to be unheard of. Protection must always be provided against the direct radiation of heat (fire-screens, lamp-shades). In a place where we are to feel comfortable in air of 20°, a vacuum thermometer must not show decidedly higher values than an ordinary thermometer. For the temperature of factories see below.

2. The air in living-rooms should, according to Deneke, not have a deficiency of saturation exceeding 5·3. Dry air, such as hot air pipes and all other heating arrangements connected with powerful ventilation often involve, may become very oppressive; a deficient saturation of eight to nine is pronounced by Deneke, the extreme limit temporarily admissible in heated rooms.

In opposition to these views other authors advocate the admissibility and even the agreeableness of much drier air at mean temperatures in dwelling-rooms. Reinhard (*l.c.*) and Ferd. Fischer (*Gesundheits-ingenieur*, 1887) pronounce a deficient saturation of 14·5 quite pleasant at + 20°; a deficiency of 10·1 to 14·3 (relative moisture from 20 to 40 per cent.) was frequently observed in the Polytechnicum at Hanover, and yet the existing appliances for moistening the air were not brought into action. Observations which I have made on myself, and on numerous other persons, agree well with these

[1] In Russia, the United States, &c., the temperature of the interior of houses in winter is kept much higher than in Britain.—*Editor.*

statements. A deficient saturation of 10 to 12 *grm.* was often observed in the laboratory at temperatures of 20°, and did not seem to me remarkably dry; other observers now and then declared that they felt a slight dryness in the throat, but experienced no other inconvenience.

I have further taken the opportunity at meetings of societies to effect changes in temperature and moisture, and afterwards to collect the opinions of men whose lives have been spent in scientific research on the proportions of water in the air. They were nearly always contradictory. Thus, of six persons in a room with a relative moisture of 50 to 60 per cent., in which the temperature (in winter) rose in ninety minutes from 17·4° to 23·6°, and the deficiency of moisture increased rapidly from 6·8 to 8·6, afterwards fluctuating between 9 and 10, two pronounced the air very dry, one as dry, two as moist, and one as moderately moist. In general, as a matter of course, a very moist air at a high temperature in closed rooms is found unpleasant, but numerical data cannot hitherto be produced. In factories the hot, damp air (40°), *e.g.*, in badly ventilated drying-rooms in dye-works or in sugar-works is exceedingly unpleasant. Dry air at 35° is easily endured if the clothing is light (*e.g.*, in rooms in which cube sugar is cut up), and even the dry, hot air (100°) of malt-drying floors can be endured very well, even without any previous habituation. For further information see Renck, and concerning warm, moist air see Stapf, *Archiv f. Anat. und Physiologie*, Supplement, 1879.

An important guide for deciding on the purity of the air of rooms is furnished by the proportion of CO_2. Pettenkofer found that air which, in consequence of the presence of human beings, or of the combustion of lighting materials, contains 1 per thousand of carbonic acid, appears to our senses as still endurable, but that, if the proportions are higher, oppression (of the nose, the general state, &c.) occurs, due not alone to the carbonic acid but to other gases simultaneously introduced. In rooms which serve for the temporary presence of many persons, as in theatres, concert-halls,

schools, &c.,[1] we must for the present be content if the proportion of carbonic acid does not exceed 2 parts per thousand, since in localities of this kind, even though well ventilated and lighted with electric lamps, it has hitherto been found scarcely possible to obtain a much purer air after an occupation of some hours. On the other hand, proportions of CO_2 of 3 to 5, and even 6 per thousand, as is still in many places unfortunately the case in public buildings (*e.g.*, in the lecture-halls of universities) must be pronounced inadmissibly and unbearably high, and improvement should be pressed for as far as possible.

To use the organic matter as a standard for the purity of the air in dwelling-houses, as proposed by Uffelmann, seems to be premature. He recommends that air should be characterised as bad if the organic matter in 1,000,000 parts requires more than 12 parts of oxygen for its oxidation; it has then a faint smell.

Hitherto it has not been found practicable to demonstrate experimentally the injurious character of human emanations (see Hermans, *Arch. f. Hygiene*, i., and K. B. Lehmann, and Jessen, *Arch. f. Hygiene*, x). At least the statements of Brown-Séquard and D'Arsonval (*Société de Biologie*, 1888) as to the poisonous character of expired air are absolutely erroneous. There is, however, no doubt that both acute and chronic injury may arise by a stay in closed localities, but a definite explanation is wanting. For acute cases (fainting-fits in overcrowded rooms), along with heat, unsuitable and tight clothing, nervous indisposition, perhaps idiosyncrasy as regards the action of malodorous emanations, must be taken into consideration. As regards chronic injuries, want of light and exercise, in addition to unsuitable nutriment, &c., play a very important part.

As to the poisonous character of numerous industrial gases there exists now for a long time no doubt. See Eulenberg, *Lehre von den Schädlichen und Giftigen Gasen*, Brunswick, 1865; and Hirt, *Krankheiten der Arbeiter*, Breslau. Unfortunately the quantitative statements of Hirt as to the concentration of gases which man can endure without injury are quite useless, being often from 50 to 100 times too high.

[1] In London the courts of justice have until lately afforded striking instances of ill-ventilated localities.—*Translator.*

TABLE OF THE CONCENTRATIONS AT WHICH SOME IMPORTANT INDUSTRIAL GASES OCCASION INJURY TO HEALTH, COMPILED FROM RECENT INVESTIGATIONS.

	Concentrations which rapidly occasion dangerous injury.	Concentrations bearable for 30 to 60 mins. without grave effects.	Concentrations which occasion only trifling symptoms after an action of some hours.	Authorities.
Hydrochloric acid gas	1·5–2 per 1000	0·05 maximum, 0·1 per 1000	0·01 per 1000	K. B. Lehmann, *Arch. f. Hyg.*, v. Matt, Dissertation, Würzburg, 1889.
Sulphurous acid	0·4–0·5 per 1000	0·05 per 1000, or less	...	Ogata, *Arch. f. Hyg.*, iii.
Carbonic acid	About 30 per cent.	6–8 per cent.	1–2 per cent.	Emmerich, Friedländer and Herter, *Zeit. f. Phys. Chemie*, ii.
Ammonia	2·5–4·5 per 1000	0·3 per 1000	0·1 per 1000	K. B. Lehmann, *Arch. f. Hyg.*, v. Matt (*l.c.*)
Chlorine, bromine	0·04–0·06 per 1000	0·004 per 1000	0·001 per 1000	K. B. Lehmann, *Arch. f. Hyg.*, vii. Matt (*l.c.*)
Iodine	...	0·003 per 1000	0·005–0·001 per 1000	Matt (*l.c.*)
Hydrogen sulphide	0·5–0·7 per 1000	0·2–0·3 per 1000	0·1–0·15 [1]	K. B. Lehmann, *Zeit. f. Hyg.*, xiv.
Carbon disulphide	10–12 *mgrm.* per litre.	2·3 *mgrm.* per litre	1–1·2 *mgrm.* [2]	K. B. Lehmann, *Bericht der Bau. Akad.*, March 3, 1888; *Arch. f. Hyg.*, xv.
Carbon monoxide	2–3 per 1000	0·5–1·0 per 1000	0·2 per 1000 not hurtful to man.	Max Gruber, *Arch. f. Hyg.*, ii.

All the experiments in columns 2 and 3, except those of sulphurous acid, carbon disulphide, and carbon monoxide, have also been made on men. Whether the concentrations in column 3 have no effect on more prolonged exposure has yet to be examined.

The presence in the air of mercury, arsenic, or phosphorus

[1] The action of 0·1 to 0·15 for six hours occasions severe subsequent illness.

[2] Exposure for six hours to 1 to 1·2 *mgrm.* occasions very unpleasant subsequent effects (headache): an exposure for two to three hours has scarcely any appreciable effects

must always be objected to. At 0°, 1 *cbm.* of air can take up 4·1 *mgrm.*; at 10°, 6·8 *mgrm.*; at 20°, 10·4 *mgrm.*; and at 30°, 16·8 *mgrm.* of mercurial vapour (Renk); but Renk only found 1 to 2 *mgrm.* in an experimental room fitted up after the manner of the silvering-room in a mirror-factory (*Veröff. des k. Gesundheitamtes*, v.). On arsenic see also section on dwelling-houses. A decision on the importance of the contamination of the air for vegetation is not included in the plan of this work; it may only be mentioned that plants, especially conifers, are exceedingly sensitive to acid vapours (HCl, SO_2), very much more sensitive than man. See Listy, *Zeit. f. Angewandte Chemie*, 1891, No. 5.

§ 152. For a decision on the constituents of dust, materials are scanty. Air containing more than mere traces of notoriously poisonous dust, preparations of lead, mercury, mercurial amalgams, &c., must be regarded as unquestionably dangerous. Hitherto it is not possible to state how small the quantities of these substances per cubic metre of air must be, in order to be regarded as harmless. For the present a very strict treatment of every such case will be suitable. For traces of lead, arsenic, and antimony in the air of printing-offices see Roszahegyi, *Archiv f. Hygiene*, iii. 522.

It is perfectly well known that air not poisonous in itself can give occasion to so-called dust-inhalation diseases. But hitherto only a few quantitative determinations of dust in the rooms of factories have been undertaken by Hesse (*Dingler's Polyt. Journal*, 1881). They may serve for general guidance.

Hesse found, in inhabited rooms, in 1 *cbm.*, dust to the extent of . . . . . 1·6–0·0 *mgrm.*

In a factory of felt shoes	160–175·0	,,
Mill (new system)	4·4	,,
Grinding-mill (old system)	47·7	,,
Rag-room (paper-mill)	3·8–24·9	,,
Cleansing-room (ironworks)	71·7–100·0	,,
Coal mine	14·3	,,
Metal mine	14·5	,,

These observations do not agree well with those of Uffelmann, who found in the air of his well-ventilated house a mean of 16·8 *mgrm.* of dust. His observations, which were made by a careful method, decidedly deserve the preference.

Among kinds of dust not specifically poisonous, coal dust seems to be the least harmful; otherwise, inorganic dust, on account of its sharp angles, is more injurious than organic matter. We cannot enter here more closely into these questions, especially as we should have to fall back upon very superficial statistics.[1]

§ 153. The number of bacteria in the air of rooms fluctuates very much. Petri found in the rooms of the Hygienic Institute at Berlin from 0 to 400 and 900 per cubic metre; Uffelmann found in his house more (2600 to 12,500), though it was well ventilated, and kept clean: in non-ventilated rooms there were as many as 165,000. French observers found very high figures in old houses and hospitals (36,000 to 79,000), whilst in more modern houses they found a number of 3480. Of pathogenic bacteria only the following have as yet been recognised: *Streptococcus erysipelatis*, *Staphylococcus pyogenes aureus* and *albus*, Friedländer's *Bacillus Pneumoniæ*.

The bacilli of tubercle must also be present in the air, as animals have often been made tuberculous by the subcutaneous introduction of dust from rooms occupied by phthisical patients (Cornet, *Zeit. f. Hygiene*, v.). Unfortunately the bacteriological examination of the air has hitherto rarely succeeded in doing practically important services, as the majority of the infectious diseases which, according to experience, are communicable by the air, depend either upon parasites as yet

[1] Much attention has been given to the injurious effects of the dust produced by grinding steel articles, which occasioned a peculiar pulmonary disease known as "grinders' asthma." It has been combated by means of magnetic respirators, and by casing the grindstones in sheet-iron jackets, traversed by strong currents of air, and provided with slits, capable of regulation, through which the blade, &c., was applied.

The dust of flax-mills and of mechanical brush-factories is exceedingly offensive.—*Translator.*

totally unknown (measles, scarlatina, typhus exanthematicus, &c.), or they are with probability referred to organisms which do not grow on the ordinary nutrient media (malaria).

The presence of the ordinary non-pathogenic aërial bacteria is evidently without any influence upon our health, even though, as it is known, the majority of the germs inspired adhere to the respiratory passages; and it seems hazardous to pronounce air rather poorer in microphytes healthier than such as contains them in larger numbers. But a constantly high number of micro-organisms may warrant the suspicion that in such premises the cleanliness of the floors is imperfect, and that in general there prevail non-hygienic conditions. The assumption is also permissible that where saprophytes flourish in abundance, and are found in the dust, a pathogenic organism may occasionally find favourable conditions for its ectogenous growth and for its presence in dust.

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SECTION II.

THE SOIL.

A. Examination of the Soil.

§ 154. The hygienic examination of the soil must in the first place reply to one of the practical questions, such as:—

Is a soil adapted for habitation, for an irrigation-field, or for a cemetery?

For the decision of these questions it is, in the first place, necessary to know the situation of the plot of land. The relations of altitude, the aspect, and the conditions in respect to moisture must be studied by personal inspection at the spot, assisted by a study of the most special maps, and with reference to the opinion of technical specialists. A conscientious examination involves not merely as accurately as possible the distribution and efflux of the surface water (lakes, swamps, or streams), but the relations of the ground-water, especially the question: How deep below the surface is the level of the ground-water, and to what extent does it fluctuate in the course of months and years? (§ 172). A further question is: Does the fluctuation of the ground-water depend on the rainfall, and can it therefore supply a measure for the moistening of the upper strata of the soil, or is it chiefly dependent upon the state of the watercourses which traverse the district openly? For a decision it is necessary to compare prolonged series of observations of the height of the rivers and the level of the ground-water.

In the second place, the local climate, in the widest sense of the word, demands our attention: temperature, direction and strength of the wind, quantity and distribution of rain, &c.

Lastly, the soil itself must be examined in its entire

character—whether we have to do with a rocky, stony, sandy, clayey soil, rich or poor in humus, how the several strata follow each other in section (geological profile), &c.

Often symptoms of the pollution of the soil are at once detected by sight and smell—*e.g.*, patches of dark infiltration in a light-coloured soil, offensive odours, &c.

§ 155. By means of the characters here intimated, the practical man generally finds it possible to arrive at a decision with the certainty which is hitherto attainable in this difficult sphere. Examinations in the laboratory may somewhat modify the results arrived at, supporting or correcting them; but hitherto, unfortunately, not very much of importance for hygienic practice has been reached from all the laborious and theoretically interesting investigations of soils in the laboratory, excepting from the quest for pathogenic microphytes. Hence we shall merely here characterise only the most common principal methods of investigation, which are:

1. The chemical composition of the soil, especially the proportion of organic matter, and, in connection herewith, the composition of the ground air.
2. The determination of the proportion of moisture, the size of the grains, the volume of the pores, and the power of absorbing water.
3. Determination of the temperature of the soil.
4. A bacteriological examination.

In order to take a specimen of soil for analysis we must, in case of a rocky ground, keep in view both the solid rock and any products of its weathering: in the far more frequent case (to be here alone more closely considered) of an earthy soil, specimens should be taken at different parts and at different depths. We dig square holes, with well-defined edges about 50 *cm.* in breadth, and we collect from them large specimens of soil, say at four depths—surface, depth of ½ *m.*, 1 *m.*, and 1½ *m.*, or perhaps even from a still greater depth. We fill by preference with each sample a 2-litre sheet-metal canister, which closes well—or, better still, a wide

glass jar with a ground stopper. If, in digging, we meet in succession with distinctly different layers, the specimens must be arranged accordingly, noting at what depth such strata are found.

1. Chemical Examinations.

§ 156. A chemical examination of the soil for the inorganic constituents of which it is constructed, presents only in few points a hygienic interest. The rock from which the soil is derived is less important as regards its hygienic properties than in its state of aggregation.

The chief inorganic constituents of the rocky and earthy soil are: silica (quartz); silicates and double silicates of aluminium, iron, calcium, magnesium, and the alkaline metals; calcium, magnesium, and iron carbonates; iron oxides; sulphates; chlorides, phosphates, and nitrates of calcium, magnesium, and the alkali metals. Among these there generally predominate either (1) silica; (2) silicates and double silicates; or (3) calcium and magnesium carbonates.

For an insight into the mineralogical composition of a soil sufficient for many purposes (especially if poor in organic matter), the following process may serve: the soil, finely pulverised, is covered with hydrochloric acid at 10 per cent.; calcium carbonate and phosphate, a part of the iron, and a few silicates which are exceptionally easily soluble, are quickly dissolved; the residue is covered with concentrated hydrochloric acid, and allowed to stand for twenty-four hours, when there are dissolved magnesium carbonate (magnesite and the magnesium carbonate of dolomite), compounds of iron, and some further quantities of readily soluble silicates, *e.g.*, chlorite ($8MgO . Al_2O_3 . 5SiO_2 . 7H_2O$); if the specimen is now boiled with concentrated hydrochloric acid, the gypsum ($CaSO_4 + 2H_2O$) and serpentine ($3MgO . 2SiO_2 . 2-3H_2O$), with some other silicates, pass into solution, but quartz and the main quantity of the silicates remain undissolved (*e.g.*, felspar, clay, hornblende). A quantitative de-

termination of these compounds *as such* is only partially possible, and only the proportion of clay is of much interest. The elements of the compounds can, of course, be readily determined according to the general rules of quantitative analysis.

§ 157. In certain cases it is desirable to determine the proportion in a soil of sodium chloride, nitric acid, nitrous acid, and organic matter, in order to show that the ground is contaminated with excreta, and with the transformation products of human existence.

For the determination of NaCl, N_2O_3, and N_2O_5, we weigh out a large sample of the fresh soil (*a g*), render it air dry, weigh again (*b g*), sift off the constituents which are finer in size than 2 *mm.* (§ 160), and contain all the salts sought for; weigh them (*c g*), and cover 500 *grm.* of them with 1 litre of distilled water in a 2-litre flask, shaking well and repeatedly. After standing for forty-eight hours the watery extract is drawn off with a syphon and filtered. The filtrate contains the chlorides, nitrates, and nitrites entirely (and generally also some sulphates). They are accurately determined as described for "water" (§ 175), the solution having first been concentrated if needful.

The calculation for 1000 *grm.* of soil is effected as follows: If in *m g* fine soil, air-dried, there are found *x g* of a constituent, *a*, *b*, *c* having the meanings given at the commencement of § 157.

$$\text{In total moist soil: } \frac{x.c.1000}{m.a}$$

$$\text{In total air-dry soil: } \frac{x.c.1000}{m.b}$$

And if the total air-dry soil still contains *p* per cent. of water,

$$\text{then in total dry soil: } \frac{x.c.1000.100}{m.b.(100-p)}$$

The remaining constituents are determined in the total soil finely pulverised. Among the inorganic constituents we are chiefly concerned with the proportion of clay ($Al_2O_3 2SiO_2 + 2H_2O$), which, above all, renders the soil compact, absorbs much water, and retains it energetically. Hitherto no exact determination of clay has been attempted for hygienic purposes; practical hygiene is satisfied at most with making a determination of the part which can be separated by elutriation and calculating it as clay, although very fine pulverulent silica and other matter is present. Agricultural chemistry has elaborated a method which depends mainly on the fact that concentrated sulphuric acid dissolves out of the soil only the alumina present in the form of "clay," and not other silicates, *e.g.*, felspar. In the

sulphuric solution aluminium hydroxide and iron are precipitated by means of ammonia, and must be further separated if the iron is present in large quantity. For every milligramme of Al_2O_3 weighed we calculate 2·53 *mgrm.* of clay. For details see Wahnschaffe. The method is not quite simple.

Every soil contains organic substances; barren sand and gravel contain very little, but arable soils, forest earth, and peat contain large, or, indeed, very large quantities. The more abundant the vegetal remains, which have become mingled with the inorganic constituents of the soil, the larger is the proportion of the so-called humous substances, brown or black acid or neutral bodies, sparingly soluble, rich in carbon, but free from nitrogen, and hitherto not fully examined by chemists. Hitherto hygiene has only attempted a determination of the organic matter in the soil in case of earth originally poor in organic matter, but here and there contaminated by the refuse of human residence (fæcal matter, drainage, &c.), when it is required to ascertain the relative degree of pollution of such parts.

For this purpose it is assumed that the organic substances all contain 58 per cent. of carbon, the average of the humous bodies. We may proceed according to any one of three methods. We may:

1. Expel all pre-existing CO_2 (§ 179*a*) by heating with dilute sulphuric acid; oxidise the organic matter to carbonic acid by treatment with potassium dichromate and sulphuric acid, and determine this carbonic acid, or we may:

2. (Only in soils containing no hydrates such as clay [$Al_2O_3 + 2SiO_2 + 2H_2O$]), determine the loss on strong ignition in the blast, consisting essentially of the organic matter along with the carbonic acid of the carbonates. If the latter is determined in another specimen we ascertain approximately from the difference (loss on ignition $- CO_2$) the weight of organic substances. The errors which attach to the analogous method in determining organic matter in water are here smaller. If the soil contains gypsum or clay, we must remember that for each molecule of gypsum or clay $2H_2O$ are expelled on ignition.

3. The most accurate procedure is to determine the pre-existing carbonic acid in one portion, and to burn a second portion carefully with potassium bichromate and lead chromate, as in an elementary organic analysis, collecting the carbonic acid. See J. A. Müller (*Chemiker Zeitung Repert.*, xv. 50).

Nitrogen is present in soils not only as nitric acid, nitrous acid, and ammonia, but in organic compounds, especially in animal waste. The nitrogen in 5 *grm.* of soil is determined according to Kjeldahl. (See "Constituents of Foods.")

§ 158. It is occasionally important (*e.g.*, in examining whether a soil is suitable for an irrigation field) to examine:

1. What is the power of a given soil to withdraw organic substances from solution, whether chemically or physically?

2. To what extent does a soil destroy or oxidise such substances?

In principle such experiments are conducted by filling wide burettes

with water up to a certain height, pouring upon them solution of urea, alkaloids, &c., of known strength, and examining the liquid which drains off below, both for a decrease of the substance originally poured upon the soil and for its oxidation products. Much depends here upon the concentration of the solution, upon temperatures, access of air, and the manner in which the liquid is poured in. (For details see Soyka, *Archiv f. Hygiene*, ii.)

The following facts give an indication without special experiments. Suspended substances are kept back best by soils of the finest texture; the alkalies potassium, sodium, and ammonium are chiefly seized by the silica of the double silicates and by the humic acids. The calcium carbonate and the ferric hydroxide of the soil absorb phosphoric acid. Nitrates, nitrites, and chlorides are least easily retained. Organic compounds of high molecular weight (colouring and odoriferous compounds, alkaloids, &c.), are retained by all sources by means of surface attraction, though least completely by those of coarse texture. Destructive oxidation is most readily effected by coarsely porous soils rich in air, with the co-operation of certain schizomycetes. High temperatures, varying through copious moisture, and slight concentration of the substances to be oxidised accelerate the process. The ultimate products are nitric and carbonic acids.

If the access of air is restricted processes of reduction—more resembling putrefaction—may predominate, also initiated by the action of certain bacteria. This state is indicated by the formation of ammonia, hydrogen disulphide, &c.

Determination of Carbonic Acid in the Ground Air.

§ 159. This investigation was formerly practised to a great extent, since, according to Pettenkofer's earliest communications (*Zeit. f. Biologie*, vii.), there seemed room for hope that the proportion of carbonic acid in the soil might be the measure of its contamination and of the organic life present. For the method see § 147. It is rarely now carried out for practical purposes, since the conditions which determine the proportion of carbonic acid in the ground-air are so complicated that single results lead to no trustworthy conclusions: it is especially striking that places situate close to each other yield constant values which differ greatly from each other. The air of the most superficial layers of soil is fairly rich in carbonic acid, that of the lower strata extremely so, a proportion of towards fifty per thousand is found at many places even at the depth of 2 *m*.

The frequently high proportion of carbonic acid in the ground-air causes it to appear desirable to prevent its access to our houses as far as possible. Other injurious gases are scarcely present in the ground-air in demonstrable proportions (sometimes a little ammonia, sulphuretted hydrogen, and hydrocarbons). The ground-air is free from microbia.

2. Examination of the Soil for its Behaviour with Air and Water.

§ 160. Every soil, rock, loam, and sand contains between its particles, whether loosely or compactly agglomerated, vacuities (pores), which are filled with air or water; they differ greatly in size and number, and the sum of their contents (the total volume of the pores) is consequently very various.

Determination of Proportion of Water.—We weigh out a large average sample of the soil (about 50 *grm.*), spread out in a capacious porcelain capsule. It is weighed accurately to a decigramme, and dried at 100° until the weight is constant. If the soil contains large stones, they are removed from a much larger weighed sample; the stones are weighed, and their moisture, as well as that of the earth, is determined separately, and the moisture of the entire soil is then found by calculation.

Determination of the Size of the Grains.—Most soils consist of fragments of different sizes. For their separation we dry the soil (about 1 *kilo.*), crush with the fingers the portions of earth which have adhered together, and place the whole in a Knops' set of sieves. This apparatus consists of six cylinders of sheet zinc, about 6 *cm.* in height, with sieve-bottoms, the meshes being of progressively decreasing sizes, and fixed together by bayonet-joints. The upper cylinder has the coarsest meshes, and beneath the lowest is a sheet-zinc dish, not perforated. We shake for a considerable time, and—

Upon the upper sieve there remain all particles more than 7 *mm.* in diameter	*Rough gravel.*
Upon the second, finer than 7, coarser than 4 *mm.*	*Medium gravel.*
Upon the third, finer than 4, coarser than 2 *mm.*	*Fine gravel.*
Upon the fourth, finer than 2, coarser than 1 *mm.*	*Coarse sand.*

Upon the fifth, finer than 1, coarser than 0·3 . . . . . *Medium sand.*
Upon the lowest dish, finer than 0·3 . *Fine sand.*

We weigh what remains upon each sieve, and express the result in percentages of the total weight. It is prudent to sift the contents of each dish once more over a sheet of paper, in order to see if the sifting is complete.

Example: The sand of the river Main at Würzburg consists of:

Coarse gravel . . .	0·7 per cent.
Medium ,, . . .	1·5 ,,
Fine ,, . . .	9·7 ,,
Coarse sand	30·0 ,,
Medium ,, ...	55·1 ,,
Fine ,, ...	2·7 ,,
Loss ,, ...	0·3 ,,

The agricultural chemists include everything finer than 2 *mm.* as "fine soil" or "fine earth," though there is as yet little agreement in their nomenclature. Kühn (see Steinriede) calls, *e.g.*, everything coarser than 5 *mm.* stones; what is finer than 5, but coarser than 2 *mm.*, he terms gravel (from 5 to 3 *mm.* coarse gravel, from 3 to 2 *mm.* fine gravel). The fine earth is again divided into sand (pearl sand from 2 to 1 *mm.*, coarse sand 1 to $1\frac{1}{2}$ *mm.*, and fine sand, finer than $\frac{1}{2}$ *mm.*); the portion capable of elutriation, consisting chiefly of clay, is earth. The elutriation is effected in a cylindrical glass, 30 *cm.* in height and 8·5 *cm.* in width. It is fitted at 5 *cm.* above the bottom with a cylindrical tubulure, $1\frac{1}{2}$ *cm.* in width, 2 *cm.* in length; the tubulure is closed. Fifty grammes of air-dried earth, freed from stones coarser than 5 *mm.*, is boiled for one to three hours in a porcelain capsule, until all the loosely agglomerated particles are disaggregated. The mass is then passed through a 2-*mm.* sieve into the cylinder; everything finer than 2 *mm.* is carefully rinsed into the glass, which is then filled with water to the depth of 28 *cm.* It is vigorously stirred with a wooden rod for about one minute, alternately to the right and the left, until the particles of soil float. After standing at rest for ten minutes, the stopper is drawn out of the tubulure, the turbid water is caught, the stopper re-inserted, and the cylinder is again filled with water to the upper neck, stirred up, and allowed to subside, but this time only for five minutes. After repeating this operation seven or eight times there remains in the cylinder merely sand, which is collected upon a filter, dried, weighed, and separated by sifting. The first and second portions of the elutriation water are evaporated down directly; the

others are collected in 2-litre glasses, and allowed to deposit. The clear supernatant water is drawn off with a syphon, whilst the deposits are all placed upon the same filter, dried, and weighed. The quantity of the portion which admits of elutriation gives a useful indication of the quantity of clay, but this method has hitherto been almost exclusively used in agricultural chemistry.

§ 161. **Determination of the Volume of Pores.**—Different methods have to be applied for rocky and earthy soils.

1. *Rocky Ground.*—We take a piece of the size of a small apple. By exposure for three hours in a drying-closet heated to 100° to 120°, its moisture is expelled, and its weight is determined $= a$. The fragment is then laid in a capsule of water, and boiled for about half an hour, until all the pores are freed from air, and filled with water. The whole is then allowed to cool, the stone is taken out, dried superficially, and weighed again. Its weight is called $= b$. Then $b - a =$ the weight of the water which has penetrated into the pores, or to the volume of the porous spaces (since 1 *grm.* = 1 *cc.* of water). In order to obtain comparable numbers we determine the volume of the stone inclusive of the pores, *i.e.*, we place upon one pan of a balance a roomy beaker half full of water, and we lay upon the other grains of shot, until an equilibrium is reached. The stone, suspended from a fine brass wire, is slowly immersed entirely in the beaker. In order to restore the equilibrium we must place upon the other scale the exact weight (c) of the volume of water taken up by the stone. The weight (c) is therefore equal to the the volume of the stone.

Now $\frac{b-a}{c} \times 100 =$ the volume of pores in per cents.

Further, $\frac{a}{c} =$ the specific gravity of the porous stone (*i.e.*, including the pores).

$\frac{a}{c-(b-a)} = \frac{a}{a+c-b} =$ the specific gravity of the mass of stone supposed free from pores.

It is more correct to use the stone first for the determination of its volume after the pores have been filled with water, rather than to make

the experiment on the stone when containing air, as in this case the air is expelled by the penetrating water only from a part of the pores. If the substance is very coarsely porous or partially soluble in water, the dry fragment is coated with a thin layer of varnish or paraffin before determining its volume.

2. *Shingly and Sandy Soils.*—For very accurate determinations (Flügge) the natural soil must be dug out into cylinders, and after it has been cautiously dried the air in its pores must be expelled by CO_2. The air expelled from the soil is collected in inverted bottles filled with strong potassa-lye, whereby all carbonic acid is absorbed, whilst the air collects and can be measured. The carbonic acid present in the ground air is here disregarded. In most cases we may be satisfied with the following approximative method (Renk):

The soil for the determination of the size of the pores, after the larger stones have been removed, is placed in sheet-metal cylinders of 20 *cm.* in height, and in width 5 to 6 *cm.* for finely granular soils, but 10 *cm.* for those of a coarse texture. The bottom of the cylinder is then closed with fine brass-wire gauze. In order to fill the cylinders as compactly and uniformly as possible, the sides of the cylinder are struck from time to time with a wooden hammer, so that the masses lie closely together. In soils where the single particles are of equal size and of a globular form, the volume of the pores may be caused to fluctuate from 26 to 47½ per cent., according as the particles lie $\begin{smallmatrix}\circ\circ\circ\\ \circ\circ\end{smallmatrix}$ or $\begin{smallmatrix}\circ\circ\circ\\ \circ\circ\circ\end{smallmatrix}$. Hereupon 500 *cc.* of water are poured into one (or two) brass cylinders, holding 1000 *cc.*, and the contents of the soil cylinder (volume v) are slowly poured in, whereby the contents are increased by the volume of the soil without pores v_1, whilst the air is expelled out of the pores.

$$v - v_1 = \text{volume of all pores.}$$

$$\frac{(v-v_1)\,100}{v} = p = \text{pore-volume in per cents. of the air-containing soil.}$$

Example: Sand of the river Main. Cylinder of 3·2 *cm.* radius, 20 *cm.* in height, and 644 *cc.* volume. If we pour the contents into 500 *cc.* of water, the volume is increased to 890 *cc.*; whence 890 – 500 = 390 *cc.*

of sand, and $644 - 390 = 170$ *cc.* of pores in 644 *cc.* sandy soil. The volume of pores amounts, therefore, to $\frac{264 \times 100}{644} = 39{\cdot}4$ per cent.

§ 162. **Determination of the Capacity for Water** (power of absorbing water). The dry soil is filled into a sheet-metal cylinder (volume v), well knocked together, and the cylinder is weighed empty (a) and full (b). The soil cylinder is placed in a deep vessel of water, so that the water extends almost to the upper margin of the sheet-metal cylinder. Sandy soils are immediately moistened through and through; clay soils not until hours, or even days, have elapsed, so that the upper surface of the soil is quite wet. The cylinder is then lifted out and allowed to drain upon a plate until only single drops ooze out. It is then dried externally and weighed (c). Now $c - b = w$, the weight of the water retained; $\frac{w \times 100}{v}$ = the weight of the water retained by 100 *cc.* of soil. From the determination of the volume of pores we know that in 100 *cc.* of soil the pores are p *cc.*

$$p : \frac{w \times 100}{v} = 100 : x.$$

x = water capacity, *i.e.*, the water retained by 100 *cc.* of soil in percentages of the volume of pores. As the retentive power depends, in the first place, on the quantity of the capillary spaces in the soil, it is a very good measure for the fineness of the pores of the soil. The finer the pores the smaller is the permeability for air. A direct determination of the permeability for air can therefore, for practical purposes, be superseded by a determination of the capacity for water.

Example: The sand of the Main. The metal cylinder of 3·2 radius and 20 *cm.* in height weighs, when empty, 198 *grm.*; when full of soil, 1224 *grm.*; after steeping in water and escape of excess, 1374 *grm.* There are, therefore, retained by $1224 - 198$ *grm.* $= 1026$ *grm.* of soil, $1373 - 1224 = 150$ *grm.* of water. From § 161 we know that the soil in the cylinder contains 254 *cc.* pores. The water capacity is therefore x $254 : 150 = 100 : x$. $x = 59$ per cent of the volume of the pores. From this example it follows that the calculation of the capacity for water is simplified if we determine it and the volume of pores by means of cylinders of equal size.

BEHAVIOUR OF SOIL WITH AIR AND WATER (RENK)

All Material from Lime Shingle Soil of Munich.	Volume of Pores in per Cent. of Total Volume.	Moistening.	Water Capacity in per Cent. of Volume of Pores.	Pressure Millimetres Water.	Permeability, Litres Air per Minute.	Loss by Moistening in per Cent. of Dry Air.	Loss by Freezing after Moistening in per Cent. of Moist Air.
1.	2.	3.	4.	5.	6.	7.	8.
Medium gravel	37·9	dry	6·6	20	15·54	...	...
		from above			14·63	5·8	5·2
		from below	12·6		13·70	11·8	10·5
Fine gravel	37·9	dry	7·8	40	14·04	...	...
		from above			13·16	6·1	5·4
		from below	16·9		12·55	10·6	19·0
Coarse sand	37·9	dry	23·4	40	2·33	...	...
		from above			1·91	18·0	14·1
		from below	31·2		1·71	26·6	25·7
Medium sand	41·5	dry	47·0	150	0·57	...	...
		from above			0·11	80·7	36·4
		from below	68·0		0·00	100·0	...
Medium sand	55·5	dry	36·4	150	0·84	...	...
		from above			0·23	72·6	100·0
		from below	46·5		0·00	100·0	...
Fine sand	55·5	dry	65·1	150	0·01	...	...
		from above			0·00	100·0	...
		from below	77·4		0·00	100·0	...

Col. 5 gives the pressure at which the values of col. 6 have been obtained. Col. 7 shows what percentage of the air which passed through the dry soil is transmitted by the same soil when moistened. Col. 8 shows what percentage of the air which has passed through the moist soil is transmitted by the same soil after freezing.

3. Examination of the Temperature of the Soil.

§ 163. A thermometer plunged with its bulb into the soil to the depth of 1 *cm.*, duly fixed and secured against breakage by wire netting, shows the temperature of the upper layer of the soil. Moist soil becomes heated more slowly, but retains its heat longer than dry ground; a dark soil becomes much warmer than an otherwise similar soil of a lighter colour. In order to ascertain the temperature of the soil at lower levels, say at ½ *m.*, 1 *m.*, and, if needful, at 3 *m.*, we insert into the earth to the depth of 3 *m.*, and in a vertical position, a wooden shaft of a square section and 3 *m.* in length. In it are placed, one upon the other, three blocks of wood, fitted at top with iron handles. The lowest block is 2 *m.* in height, the intermediate and the upper block each ½ *m.* high. Each block has at its lower end, in one of its sides, a groove

in which is placed a sluggish thermometer, the bulb of which, when the block has been sunk into the shaft, is exactly at the depth intended. Opposite the bulb of each thermometer the wooden shaft is interrupted by a short horizontal copper tube, which, as a good conductor of heat, easily permits the temperature of the soil to find access to the corresponding thermometer.

Every twenty-four hours, or more frequently, the ther-

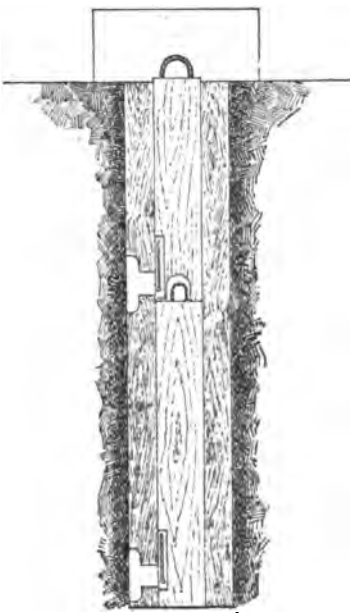

FIG. 82.

mometer blocks are drawn up by the hand, or the deeper ones by means of a hook thrust into the handle, and the thermometer is at once read off. As in Europe the laws of the distribution of the temperature of the soil are known with sufficient accuracy for practical purpose, no such investigations need now be undertaken. (See Soyka, *Der Boden.*)

4. Bacteriological Examination of the Soil.

§ 164. The qualitative examination is conducted by the plate method with regard to the indications laid down below

for the quantitative method. Among pathogenic organisms there have been hitherto found in the soil the bacilli of tetanus, of malignant œdema, of splenic fever, and *Staphylococcus pyogenes albus.*

Lartel has isolated from the mud of the Lake of Geneva, where the water is 40 to 45 *m.* in depth, the exciting organism of malignant œdema, of tetanus, as also *Staphylococcus pyogenes aureus,* the bacillus *Coli communis,* and, it is asserted, that of typhus. (*Centralblatt f. Bakteriologie,* ix. 709.)

The demonstration of disease germs is effected more readily by inoculating susceptible animals (guinea pigs), § 74, with small portions of soil than by culture, which, on account of the vast predominance of saprophytes, always encounters difficulties. The bacilli above-named, if present, may thus be readily found. Cholera bacteria have hitherto not been found in the soil; they have, indeed, been little sought for. Typhus microbes cannot be recognised by experiments upon animals; and in plate cultures they are easily overlooked among the masses of saprophytes, especially when they occur sparingly. Recently Macé alleges that he has discovered typhus bacilli in the soil of a typhus locality (see § 200). Soils cannot hitherto be closely examined for the exciting cause of malaria from the want of proper methods. But we may infer the presence of the malaria organism in certain soils from the fact that it can occur in localities hitherto healthy after disturbances of the soil.[1]

The quantitative examination of soils for schizomycetes is effected as follows: As long as we have to do merely with superficial layers of the soil, or such as can be made accessible by digging, we have simply to transfer portions of the soil in question into sterilised glasses closed with plugs of wadding, using an iron spoon sterilised in a flame. But if deeper, less accessible layers of earth have to be examined, we make use of the earth-borer indicated by Fränkel (*Zeit. f. Hygiene,* ii.).

By an ingenious but simple arrangement it is possible at a required depth to draw back a sheath, so that a cavity can be

[1] Engineering operations, earthquakes, &c.—*Translator.*

filled with the required earth; if the sheath is pushed back, the borer, when filled, can be withdrawn without fear of contaminating the specimen with superficial materials. After the sheath has been withdrawn the sample of soil is removed from the groove by means of a sterilised metallic instrument (a rod, a small spoon, &c.), and transferred to a sterilised flask closed with wadding. Thereupon the borer, especially the groove and the sheath, are thoroughly cleaned with blotting-paper, and further samples may then be bored out in the same manner.

Examination of the Samples.—As soon as possible after they have been obtained, the specimens must be examined by the method of plate cultivation, especially those rich in nutrient matter obtained from the deeper strata, which are often the seat of a rapid and very considerable increase of microbia.

We measure out with a small, hemispherical, sharp-edged spoon, of steel or platinum, a very small quantity ($\frac{1}{50}$ *cc.*) of the soil, slightly crushed if needful with the aid of the instrument, and place it at once in a small tube with liquefied gelatine, in which it is farther crushed as far as possible by means of a strong platinum wire. The bacteria or the fragments of earth are then uniformly distributed, and the whole is either poured into a box or made up into an Esmarch roller-plate. Some check-plates should always be made up at the same time.

The enumeration of the microbia must take place within forty-eight hours, on account of the numerous liquefying species, and their rapid increase. As the colonies are still small, a lens must be used, and much care must be taken in counting.

The method described yielded in C. Fränkel's hands the best results; better especially than floating the fungi of the sample of soil in water, and making up the plates with this.

Eberbach found at Dorpat soils so rich in microbia (500,000 on the average) that he found it useful to dilute the specimens of soil with sterilised sand before preparing the plates.

If we wish to ascertain whether permanent forms (spores)

are contained in the soil in question, the charged tubes are submitted for an hour to a temperature of 80° before the roll-plates are made up. Anaërobic microbia are rare.

Results of the Method and their Interpretation.—In this matter Fränkel found in the upper layers of soil down to about ¾ *m.* a very high number of micro-organisms (100,000 to 350,000 in 1 *cc.*[1]), as well in cultivated as in uncultivated soils (rich or poor in vegetation). If we penetrate deeper into the soil, the proportion of microbia generally falls strikingly and suddenly off at the depth of 1, 1½, or 2 *m.* (down to 2000 to 200). Very often no micro-organisms are found at all after a certain depth, a number worth mentioning rarely occurring below 3 to 4 *m.* In cultivated land the results are rather more irregular, on account of the disturbance of the soil, the contamination of the subsoil by fungiferous sewage, &c.; still, they are substantially the same.

None of the organisms recognised by C. Fränkel are pathogenic,[2] with the exception of *Bacillus œdematis maligni.* Bacilli predominated in the superficial layers;[3] cocci were more rare, and in the subsoil there occurred chiefly hyphomycetes, especially a brown kind, which has not yet been more closely described. From these results it follows unfortunately too plainly that, for the present, we have little prospect of obtaining a certain basis for a more definite hygienic decision on a soil by an enumeration of microbia than we otherwise possess. Certainly it will appear very hazardous to infer with certainty the peculiarly dangerous character of a soil from a very high number of microbia in the superficial layers. A bacteriological examination of

[1] It must here be mentioned that Beumer at Griefswald, and Maggiora at Turin, obtained far higher numbers than Fränkel, partly because they did not at once work up the material obtained. Beumer found up to 45 millions, and Maggiora up to 78 millions; but the former has found in 1 *grm.* 1½ million, in specimens examined immediately.

[2] Der *Tetanus bacillus* war damals noch unbekannt.

[3] As regards the bacteria of the soil see also Globig, *Zeit. f. Hygiene*, iii., where a number of kinds having a very high optimum temperature are described (50° to 60°), as also resistant spores. Further, Adametz, *Pilze der Ackerkrume*, Leipzig, abstracted in *Centralblatt f. Bacteriologie*, i. 8. See also Bibliography for Water.

the soil might perhaps serve for proving a deficiency in the structure of tanks and sewers in the subsoil, if at suspicious points an exceptional number of microbia are found, as compared with their rarity in neighbouring soils. Here, however, chemical methods and simple inspection may be often as accurate and convenient.

Investigations as to whether a soil favours the development of bacteria, or favours it more than some other soil, can give results only in case of widely different soils, on account of the manifold factors which come here into play, from reasons similar to those which have shaken confidence in the practical value of the determination of carbonic acid in the soil. In pure marble sand Manfredi and Serafini (*Archiv f. Hygiene*, xi.) found a decidedly more favourable development of the bacteria of splenic fever and cholera than in quartz sand—the size of the granules, the proportion of water and of nutrient matter, being equal. The low thermic conductivity of marble, or the better preservation of the heat generated by the bacteria, is supposed to be the cause.

B. Decision on Soils.

1. Decision on a Soil as a Site for the Erection of a House or of a City.

§ 165. Where there is freedom of choice we should prefer—

1. Ridges of hills and gentle slopes, rather than valleys and steep declivities. In elevated localities the soil and the houses are drier, as more exposed to the wind, which effects a more complete exchange of air, removing smoke and harmful gases. Valley bottoms are mostly badly drained, consequently swampy, and there is deposited in them a quantity of decomposable matter from the surrounding heights. Especially in epidemics of cholera there have often appeared significant differences in different situations.

2. We should prefer westerly, southerly, or easterly exposures to a northerly aspect. In our climate—leaving the north out of the question—a westerly frontage receives least

sunshine, a southerly the most, whilst an easterly aspect takes an intermediate rank.

3. Dry sites are preferable to those which are moist, whether the moisture is derived from bad superficial drainage, from too great a height of ground-water, or by the occasional overflow of a river. The foundations of houses should always lie above the highest level of the ground-water. Malaria is especially prevalent in damp regions (it appears that the germ of malaria is not necessarily of human introduction), as also dysentery, articular rheumatism, and cerebro-spinal meningitis. Even tuberculosis, it is now established, can be decreased by the drainage of the damp subsoil of a city. On the other hand, the Bavarian moorlands, which suffer from malaria, have a striking immunity from cholera.

4. Taking into consideration that in polluted soil (rich in nutrient matter), containing in its pores abundance of air and water, and possessing a sufficient temperature, pathogenic microbia multiply, and thus pass from the soil into the houses, and then directly, or indirectly, into man, we must recommend as the most suitable ground:

a. Solid rock, slightly porous, free from chinks and from the infiltration of filth. Many rocks are originally soft and porous, and as liable as earth to the infiltration of polluting matter (*e.g.*, the notorious rocks of Malta); others are shattered, the crevices being filled with rubbish (*e.g.*, Karst).

b. Pure sandy and pebbly soils. The poorer such soils are in organic nutrient matter, and especially the drier they are, the less is the probability of their affording a nidus for the growth of pathological organisms. We must not conceal the fact that it has not been experimentally proved with incontrovertible certainty that the germs of the disease multiply in the open soil, and produce epidemics by passing from the soil into man.

For malaria this is universally conceded, without direct proof: for typhus and cholera also an epidemiological signification of the soil can scarcely be doubted, though in the absence of direct bacteriological evidence. Attention

must here be called to the facts which have been collected by the unwearied researches of Von Pettenkofer, which prove a local and seasonal predisposition to typhus and cholera, though they cannot be here discussed.

It seems scarcely possible to explain these facts without the assumption that the local predisposition finds its explanation in the character of the soil, whilst the seasonal predisposition depends on the varying moisture, heat, &c., of a predisposed soil. At least no other hypothesis attempted possesses even an approximately equal probability. The fact of winter-epidemics in the severest cold seems to show that the open ground is at least not the sole place of the ectogenous increase of the pathogenic bacteria concerned.

De Giaxa (*Annales de Micrographie*, 1890) has studied the behaviour of the cholera bacillus in the soil: whilst it increases rapidly in sterile soils, or in those poorer in saprophytes, and survives for two to three weeks, it perishes in soils rich in nutrient matter, and not sterilised in even two, or at the most four, days. Concerning the typhus bacillus, Karlinski states, in a preliminary communication (*Centralblatt f. Bakteriologie*, ix. 434), that the bacilli disappear in one or two weeks only, if the ground is thoroughly moistened with rain; in other cases he has observed them remaining alive for five months.

In moist (wet) soil the eggs of *Ankylostomum duodenale* and *Trichocephalus dispar* develop into larvæ which attain their full growth in man.

2. Decision on a Soil for an Irrigation Field.

§ 166. For a plot of ground to be suitable for the reception of the waste waters of a city (generally including excrementitious matters), it must possess the following properties:—

1. It must consist of permeable soil with a moderate capacity for water. Sandy soils are the most suitable. Gravel soils retain too little water. Clay soils are useless, on account of their great capacity for water, and their complete non-permeability.

2. The plot of land must be large enough. About 20 *cbm.* of sewage are daily conveyed to a hectare of sandy soil on the irrigation fields at Berlin, *i.e.*, 2 litres per square metre.

3. The plot of land may be quite poor in organic matter; the plants obtain sufficient nourishment from the dilute sewage.

4. The greater the power of the soil to retain and oxidise organic matter, the better.

5. The plot must be perfectly drained, and a suitable outflow for the drainage must be provided.

6. The irrigation must not be too near human habitation, since it may to some extent give occasion to the development of unpleasant odours. Influences injurious to the health of the numerous inhabitants of the irrigated districts have, on careful scrutiny, not been observed in Berlin.

3. Decision on a Plot of Land as a Cemetery.

§ 167. In the soil the bodies should disappear (decay) as rapidly as possible, excepting the bones, without our senses recognising anything of the process.

To this end it is necessary that—

1. The highest level of the ground-water in a churchyard, with a depth of 1·5 to 2 *m.* for the graves, must not approach the surface nearer than 2·5 to 3 *m.* The greatest enemies of the process of decay are excessive water and exclusion of air. Then ensues putrefaction, or even the formation of adipocire, which latter process converts the corpse into a form very difficult of destruction.

2. The capacity for water must not be great. Decay is most rapid in gravel; then follows coarse sand, then fine sand; the most unsuitable is clay. Alternating moisture from rainfall, *i.e.*, a soil occasionally moistened but not wet is favourable. In a too coarse drift the excavation of the soil is difficult. Rocky soil is, of course, quite unsuitable.

3. Plains (especially elevated plains), or gentle slopes, are better than steep declivities. In such it may easily happen that the bodies in the upper part of the slope lie too dry, as

the rain-water runs off too rapidly, so that mumification is threatened. On the other hand, in the lower part of the slope the quantity of water may be too great.

As regards the danger of the vicinity of a cemetery in inhabited quarters, after a long period of indifference (masses of graves in the narrowest space, in churches, vaults, &c., in the centre of cities) exaggerated conceptions of the great danger have become general.

A cemetery properly laid out gives off no corpse gases, only if the graves are used again and again in a too rapid rotation offensive odours may arise in consequence of remnants of substances still capable of putrefaction being brought to the surface. The corpses certainly pollute the soil, but if the ground-water does not occasionally rise into the graves it remains pure and of a good taste, as it is proved by numerous analyses.

Pathogenic microbia survive only a short time in the bodies, generally eight to fourteen days, more rarely three to four weeks; better at low than at high temperatures; particularly when the corpse putrefies in water. If by any chance the formation of spores takes place splenic fever may certainly persist for years. See Von Esmarch, *Zeit. f. Hygiene*, viii. See also Petri, *Arbeiten des Kaiserlichen Gesundheits-Amtes*, vii. On the other hand, Karlinski has obtained from the spleen of a buried typhus patient, after three months, bacilli capable of being cultivated (*Centralblatt f. Bakteriologie*, ix. 434). But surviving microbia could find their way into the ground-water only in exceptional cases (the formation of crevices, &c., in the soil), the filtering power of the earth proving generally a complete protection.[1]

[1] It may be questioned whether the author does not under-estimate the dangers of cemeteries. Pasteur has proved that pathogenic matter is carried up to the surface by earth-worms, and Slater shows that it is then distributed by insects.—*Translator.*

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Steinriede, F. *Anleitung zur Mineralogischen Bodenanalyse.* Leipzig. 1887. Very thoroughgoing. Employs all the methods of mineralogy. Much original research.

Wahnschaffe. *Anleitung zur Wissenschaftlichen Bodenanalyse.* Berlin. 1887. Method clear and comprehensive, with especial reference to agricultural chemistry. Necessary for more profound studies. Fifty-seven illustrations. No criticism.

2. Works Furnishing Materials for a Decision on Soils.

Soyka. *Der Boden (Band von v. Pettenkofer and v. Ziemssen, Handbuch der Hygiene).* Leipzig. 1887. A most complete critical conspectus of our knowledge of the soil. Recognised as an excellent work.

Soyka. *Boden (Artikel in Eulenbergs Realencyclopädie).* Brief, devoted chiefly to the practical phase; extensive collection of practical experience on the influence of the soil upon epidemics. Much bibliographical information.

Wolffhügel. *Ueber die Verunreinigung des Bodens durch Strassenkanäle, Abort- und Düngergruben (Zeit. f. Biologie*, xi.).

Von Pettenkofer. *Zum Gegenwärtigen Stande der Cholerafrage (Arch. f. Hygiene*, v., vi., vii.), and in separate reprints. Displays, with numerous instances, and with abundant bibliographical references, the present views of Von Pettenkofer on the significance of the soil for typhus and cholera.

Schuster. *Beerdigungswesen.*
Flügge. *Anlage von Ortschaften.* } Leipzig. 1882. In Von Pettenkofer's and Von Ziemssen's *Handbuch der Hygiene*, II., i. 1.

Wollny. *Forschungen auf dem Gebiete der Agrikulturphysik.* Copious journal with much matter of hygienic interest. Since 1877.

Works in the English Language.

The writings of Dr. George Walker (familiarly known as "Churchyard Walker").

SECTION III.

WATER.

A. Examination of Water.

§ 168. From the most varied reasons the hygienist may be be called upon to pronounce a decision upon a water. The following are the chief points to be considered in such an examination:—

I. *Sampling and Physiological-Physical Preliminary Trials.*
   1. Taking the sample of water.
   2. Examination by sight, smell, taste.
   3. Examination of a spring for its temperature and its yield.

II. *Chemical Examination* (*Qualitative and Quantitative*).
   1. Residue on evaporation, and loss on ignition.
   2. Acids.
   3. Bases.
   4. Organic matter.
   5. Collation of results.

III. *Microscopical Examination.*

IV. *Bacteriological Examination.*

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I. *OBTAINING THE SAMPLE AND PRELIMINARY EXAMINATION, PHYSIOLOGICAL AND PHYSICAL.*

1. Obtaining the Sample.[1]

§ 169. Water from a pump-well for examination must always be collected after prolonged previous pumping (to the

[1] For obtaining water for bacteriological purposes, see § 200.

extent of 100 litres), since water which has stagnated in the pump-pipe, is often richer both in chemical impurities (especially ammonia), and in micro-organisms, than such as is taken afterwards. Water from mains is generally allowed to flow away for a while, though the first portions must be at once examined if the presence of heavy metals is in question. Water from open water-courses, canals, &c., is taken by immersing the bottle (with its bottom turned against the direction of the current) to the depth of 20 to 30 *cm.*, and then inverting it. Samples from specified depths in a shaft, sea, &c., are best taken according to Lepsius (*Tiemann-Gaertner*, p. 33).

In general 2 litres of water suffice for a chemical examination. The water is received in bottles of white glass, which have been most carefully cleansed, and then rinsed out a few times with the water in question. Errors may be occasioned by residues of detergents, alcohol, acids, grains of shot, &c., which have remained in the bottle. The bottles are closed either with new corks, which have been previously strongly boiled, and, after being dried, are plunged into melted paraffin: after being driven in, they are cut level at the top, and sealed. It is better to use bottles with ground glass stoppers, which are retained in their places by being tied down with parchment-paper. Each bottle is labelled, and, if practicable, to guard against any confusion from the displacement of the labels, they may be marked with a diamond with a number, the meaning of which is carefully noted down. If water is to be examined for gases (O, CO_2), the samples must be taken with especial precautions, in bottles filled up to the stopper, and which have previously undergone a prolonged rinsing with the water.

If water cannot be immediately examined, it must be kept in a cool place (cellar, &c.), or preferably in an ice-closet. If this precaution is neglected, further changes may take place in the constituents of the water, especially in such as contain nitrogen (see § 200).

2. Examination by the Senses.

§ 170. 1. To ascertain the smell of water, it is heated to about 50° in a large flask, which is about half filled. The odour is detected most plainly on shaking the flask round.

If we suspect a smell of sulphuretted hydrogen, we add a little of a solution of copper sulphate. The appearance of a blackish coloration, and the cessation of the smell, in consequence of the formation of CuS, confirm the suspicion, whilst an odour of mouldiness or of excrementitious matter is persistent. For a more delicate test for sulphuretted hydrogen, see § 186.

2. In order to examine the taste, the water is taken fresh; if it is very warm or cold, it is brought to from 10° to 12°. Contamination due to salts of iron, coal-gas, or to products of mouldiness and putrefaction, and large quantities of common salt, are easily tasted. In the recognition of the hardness (see § 191) of water, different persons are very differently qualified. In special experiments made for this purpose, I have often seen very soft and very hard waters (from 0·3 to 33 degrees of hardness) respectively mistaken. The common assertion that the presence of air and carbonic acid renders water pleasant to the taste, whilst nitric and nitrous acids impart a certain piquant freshness, lacks as yet a strict demonstration; certainly all these factors take a secondary place as compared with the temperature of the water. I hope shortly to present more accurate communications on this question.

3. *Optical Examination.*—Pure water, and such as contains only colourless salts, are, as it is well known, in shallow layers colourless, but of a bluish colour in deep masses. Dissolved bodies generally colour water yellowish or brownish (sewage, humic substances), other colours rarely (waste waters from dye-works). The most important suspended bodies are: clay (yellowish or greenish), ferric hydroxide precipitated by the action of the air (reddish brown), sulphur (yellowish white), calcium carbonate (white), metallic sulphides (black), insoluble humic bodies from moorlands

(brownish black), flocks of aquatic schizomycetes (crenothrix, cladothrix, beggiatoa), whitish or brownish. See § 88.

In dubious cases we may ascertain the presence of an abnormal colour by pouring the water into a tall cylinder of glass wrapped round with paper (*e.g.*, a litre measuring cylinder), which is held over white paper, along with a similar cylinder filled with pure water. On looking through the two columns of water, each about 40 *cm.* in depth, any moderately appreciable difference in colour is recognised. This method will generally suffice for practical purposes.

This may be made more sensitive if, instead of the glass cylinders, we use a brass tube 1 *m.* in length, blackened within, and provided below with a window of colourless glass. The tube stands on three feet, about 10 *cm.* in height. We look towards a sheet of paper illuminated by the sun. Brown colorations have been quantitatively estimated by a colorimetric comparison with a solution of burnt sugar (caramel). It is not unfrequently observed that water which was clear when drawn becomes rather quickly turbid on standing. This is a quite common phenomenon in water from deep wells in the low plains of North Germany. From the ferrous carbonate, which is here rarely quite absent, ferric hydroxide is separated out by the influence of the oxygen, if the water is exposed to the air. Water rich in bicarbonates, if allowed to stand in open vessels, deposits calcium carbonate as a crystalline film.[1]

In order to decide whether the colour of a water is derived from dissolved or from suspended matter, a filtration must be effected. If we use a small, dense filter, which has been previously weighed, the quantity of the deposit, when dried, can be determined by a second weighing. If the filter and the residue are afterwards incinerated, we find, on deducting the ash of the filter, the quantity of the inorganic residual constituents. Extremely fine suspended matter often passes even good filters.

If water is not clear, all chemical examinations must be preceded by a thorough filtration. In general, only the filtrate is further examined as "water."

[1] See R. von Wagner's "Chemical Technology," English version, edited by W. Crookes, F.R S., p. 228.—*Translator.*

3. Examination of a Well for Temperature and Copious Yield.

§ 171. From the nature of the case, these determinations cannot be undertaken in the laboratory, but must be effected at the place concerned, and, if at all practicable, repeatedly, in dry and wet, and in hot and cold weather.

If the water is open to the light, the temperature is simply determined by immersing a thermometer. If temperatures have to be determined in the shafts of wells, &c., it is preferable to use thermometers of the following construction: the bulb containing the mercury is enclosed in a metal cup, which, on immersing the instrument, becomes filled with water, and remains full whilst it is drawn out, so as to prevent any change in the height of the mercury. A similar protection against a change of temperature can be improvised by wrapping cotton wool round the bulb, and securing it with thread.

To test the yield of a spring is generally the duty of the industrialist. In small springs the water yielded from minute to minute may be caught and measured; in case of large springs, the section of the well-stream and the rapidity of the current must be determined with a "Weltmann's wing" at several places. A simple calculation (analogous to that employed in calculating ventilations) gives the flow of water per second or per minute. The quantity is always expressed in minute-litres or second-litres: 400 second-litres water mean, *e.g.*, that 400 litres of water flow per second.

The hygienist is sometimes required to determine the yield of a pump-well. This question may be solved by ascertaining how much water may be pumped up hourly without a considerable reduction of the water-level. If a useful result is to be obtained, the pumping must be continued for some hours, the well being sounded from time to time. In order to determine the quantity of water raised, it is best to make use alternately of two wooden casks, which are filled alternately, and which have been previously gauged by filling them with water from small measuring-vessels. Such experiments are occasionally undertaken with steam-pumps in

trial-wells specially arranged, if there is a question of supplying a town with ground-water.

§ 172. In order to determine the level of the water (ground-water) in a well with accuracy and trustworthiness, we proceed as follows, according to Pettenkofer: At the end of a sufficiently long measuring-cord (in general 10 *m.*), graduated in decimetres, hangs a rod of brass, fitted at intervals of 1 *cm.* with small, flat, horizontal cups (Cup apparatus, Fig. 83).

Fig. 83. —Cup apparatus.

The measuring-line is cautiously uncoiled, and let down into the well with the cup apparatus first, after the plate of stone or iron which closes the shaft has been removed (Fig. 84, *d*). We observe at the bottom of the shaft a small, shining, quiescent surface of water, which suddenly takes a tremulous motion as soon as it is touched by the point of the cup apparatus. From this moment the measuring-cord is lowered only until the upper margin of the opening of the shaft coincides with the nearest decimetre mark of the cord. The cord is then drawn up, and the number of metres and decimetres showing the depth of the well is read off: to the value thus ascertained is added the piece of the cup apparatus which projects above the water-level, *i.e.*, as many centimetres as cups have remained unfilled.

If, *e.g.*, we have read off 3·6 *m.* on the measuring-cord, and two cups have remained unfilled, the level of the water would be 3·62 *m.* below the surface of the earth.

An inexperienced operator sometimes commits the error of overlooking the movement of the surface of the water, which is often not easily perceptible, and immersing the measuring-cord so deeply that the entire cup apparatus is under water, and even the measuring-cord is wetted. Such a measurement is, of course, useless, and must be repeated after the cups have been emptied out. In general, the inexperienced observer will do well always to make two or three successive determinations, and depend upon them only if they agree.

For accurate and repeated observations of ground-water the measuring-band must be checked from time to time, as the slips of leather or wax-cloth used vary considerably in length in course of time.

If a well is to be arranged for permanent observations of the ground-water, this is best effected as follows (Fig. 84):—

Upon the surface of the water is placed a flat case of thin sheet brass (*a*) 20 *cm.* in diameter and 3 *cm.* in height, which acts as a float.

This case is secured to a chain (*b*), which runs above over a pulley, and supports at its other end a suitable weight (*e*), from which there projects a finger (*f*). If the ground-water rises the float is lifted and the finger falls; if the level of the water falls the result is the inverse. It is, therefore, merely necessary to fix a scale opposite the finger, and to take care that the finger is placed correctly at the beginning, when the apparatus is always ready to be read off. The first adjustment of the finger is effected by measuring the level of the water with the cord and cup apparatus as above, and varying the position of the finger by altering the length of the chain, and displacing the finger until it indicates the point of the scale which was found by reading off the measuring. Of course the apparatus must occasionally be verified by means of the measuring-cord.

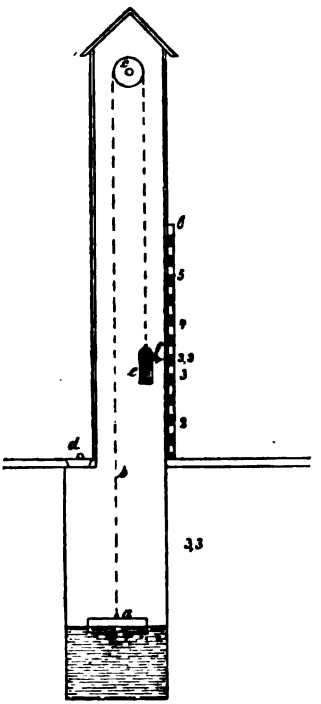

FIG. 84.—Well for Observing Ground-Water.

In general, the arrangement for reading off is locked up in a kind of closet, the door of which is opened only for observing the finger; in this manner any injury to the scale and the chain by the influence of the weather or by accident is avoided.

II. *CHEMICAL EXAMINATION.*

1. Residue on Evaporation and Loss on Ignition.

LOCALITY.

§ 173. Water without solid ingredients does not occur in nature. Rain-water is extremely poor in mineral matter. For the influence of the geological formation upon the composition of spring waters, see § 202. Refuse matter from human residence is often found abundantly dissolved in water.

QUANTITATIVE DETERMINATION.

We measure off in a measuring-flask 500 *cc.* of water (or only 250 *cc.* if the residue is large); we weigh a capsule of platinum or of thin porcelain holding about 60 *cc.*, and pour into it 50 *cc.* of the water. The water is first evaporated down according to the directions in § 1 upon an asbestos plate, avoiding violent ebullition, and adding constantly a fresh supply from the stock of water measured off for evaporation. When the volume of water is much reduced (to about 20 *cc.*) it is evaporated to dryness on the water-bath, and the residue is kept for three hours at 100° in the desiccating stove, weighed, dried again for three hours more, and weighed again. If still no difference of weight appears this operation is repeated a third time. The difference of the weight of the capsule multiplied by 2 (or 4) shows the dry residue in 1 litre of water.

Formerly it was often customary to dry at 180°, at which temperature the residuum is partially decomposed. This high temperature is still recommended if we wish to determine the organic substances by subtracting all the inorganic matters as separately determined from the weight of the dry residue (see § 174). A temperature of 180° must also be selected in case of gypsiferous water, as at 100° gypsum is separated out with two molecules of water of crystallisation, which are expelled only at 180°. Or for every milligramme of SO_3 we must deduct 0·45 *mgrm.* of crystalline water from the residue as obtained at 100°. In every case the temperature at which the water has been dried out is to be recorded.

§ 174. It was formerly the universal practice to ignite the dried residue and to consider the permanent portion, the so-called residue on ignition, as inorganic matter, and the loss of weight as organic substance. At present a smaller value is attributed to a knowledge of the loss of weight, since, in addition to the organic substances, inorganic bodies undergo changes; thus carbonates, nitrites, nitrates, sulphides, &c., are partially decomposed, alkalies are volatilised, &c.

It is, however, still important (as a preliminary test) to ignite the residue left on evaporation. If it remains white, the water is very poor in organic matter; if it becomes yellow or brownish, but easily becomes white on prolonged

heating, the organic matter is still scanty. If the colour is of an intense deep brown or black colour (due to carbon), which becomes white only on prolonged ignition, the water is to be characterised as rich in organic substances. A great loss of weight is also a proof of the presence of much organic matter. In gypsiferous waters the dry residue melts first with an escape of water of crystallisation. A loose, light, white residue indicates much magnesium.

2. Chlorine, Hydrochloric Acid, Chlorides.

OCCURRENCE.

§ 175. Chlorine is found in nearly all waters, generally combined with sodium, more rarely with potassium, calcium, and magnesium. Free chlorine is found only in industrial waste waters, and that but very rarely.

QUALITATIVE DETECTION.

For the recognition of chlorides we acidulate about 10 *cc.* of the water under examination with a few drops of pure nitric acid (free from chlorine), and add a couple of drops of a solution of silver nitrate. If chlorides are abundantly present, there is formed a white, curdy precipitate; if the chlorides are only small in quantity, there is merely a white turbidity or opalescence from silver chloride:

$$NaCl + AgNO_3 = AgCl + NaNO_3.$$

The addition of nitric acid must not be omitted, since silver nitrate precipitates also carbonates and phosphates, which, however, are soluble in nitric acid. The complete insolubility of silver chloride in nitric acid, joined to its solubility in ammonia, gives certain proof of the presence of chlorides.

QUANTITATIVE DETERMINATION.

Principle.—For this purpose the method of Mohr is available; this, if certain simple precautions are observed, ranks among the most delicate as well as the simplest processes of chemistry.

If to a neutral liquid which contains chlorides, and at the same time neutral potassium chromate, we add a solution of silver nitrate, we observe, as soon as the last molecule of alkaline chloride has been transformed with silver nitrate to white silver chloride, a permanent transposition of potassium chromate and silver nitrate to a reddish brown silver chromate. At first, therefore, and as long as a molecule of alkaline chloride is present, the reaction occurs:

$$NaCl + AgNO_3 = NaNO_3 + AgCl.$$

Afterwards the reaction is:

$$K_2CrO_4 + 2AgNO_3 = 2\,KNO_3 + Ag_2CrO_4.$$

Preparation of the Standard Liquid.—We prepare a decinormal solution of silver[1] containing 16·97 *grm.* silver nitrate per litre, of which each cubic centimetre represents 3·54 *mgrm.* chlorine.

One molecule $AgNO_3$, according to the above formula, is equivalent to one atom of chlorine; therefore $107{\cdot}7 + 14 + 3 \times 16 = 169{\cdot}7$ *grm.* $AgNO_3$ are exactly equivalent to 35·4 *grm.* chlorine; or 1 *cc.* decinormal solution of silver = 3·54 *mgrm.* chlorine.[2]

Silver nitrate occurs pure in commerce, and remains good if preserved in well-stoppered bottles, coated externally with black asphalt varnish.

Execution of the Process.—For titration we colour 100 *cc.* of water a pale yellow with three drops of the neutral solution of potassium chromate. We then add carefully the solution of silver drop by drop, stirring with a glass rod. At first the addition of the silver solution to the pale yellow liquid causes a white turbidity, which seems to be of a

[1] Any one who works much will, instead of this commonly used solution, employ a solution of silver nitrate containing 4·793 *grm.* per litre, of which 1 *cc.* = 1 *mgrm.* chlorine.

[2] To verify the solution of silver we prepare a decinormal solution of sodium chloride, *i.e.*, we place about 10 *grm.* of the purest sodium chloride in a porcelain crucible, with a cover, over a small flame, and heat until all occluded water has escaped with decrepitation. After it has been cooled in the exsiccator, 5·840 *grm.* are weighed out. 10 *cc.* of the solution must exactly represent 10 *cc.* solution of silver.

greenish white. On the further addition of silver nitrate the turbidity increases, and at last the drops of silver nitrate begin to produce a reddish brown colour, which at first disappears completely on stirring, but finally—and at this moment the titration must cease—becomes slightly permanent. We know then that all the alkaline chloride has been exactly converted into silver chloride. If, *e.g.*, we have consumed 0·7 *cc.* solution of silver, there are in 100 parts of water 0·7 × 3·54; in 1000 water, 0·7 × 35·4 *mgrm.* chlorine. If the proportion of chlorine in the water is very small, it is best to evaporate 200 *cc.* down to 100.

3. Sulphuric Acid (Sulphates).

OCCURRENCE.

§ 176. In most waters as traces, but abundantly in water derived from gpysiferous formations. Sulphuric acid is commonly combined with calcium, rarely with alkalies.

QUALITATIVE DETECTION.

The water is mixed in a test-glass with solution of barium chloride, a little hydrochloric acid having been added. A precipitate of barium sulphate, insoluble in hydrochloric acid, is certain proof of the presence of sulphuric acid:

$$H_2SO_4 + BaCl_2 = BaSO_4 + 2HCl.$$

If the addition of hydrochloric acid is omitted, white precipitates may also be occasioned by carbonates, phosphates, &c.

QUANTITATIVE DETERMINATION.

Principle.—All the sulphuric acid is converted into barium sulphate, which is dried and weighed.

Execution.—Accordingly, as in the qualitative test, as we find a faint turbidity, or a decided reaction, we operate upon 1000, 500, or 250 *cc.* of water. If we require 1000 or 500, we evaporate down to about 100 *cc.*, after acidulation with a little hydrochloric acid. For precipitation we heat the solution in a thin-sided beaker, set upon a wire gauze, to slight ebulli-

tion, after acidifying with a little hydrochloric acid, and we then add slowly a hot dilute solution of barium chloride (saturated solution, diluted with from five to ten volumes of water), stirring constantly, whereby the clear liquid becomes turbid. More and more of the above solution of barium chloride is gradually added, until on a further addition no increase of turbidity appears in the water. In water containing very much sulphuric acid we may be in doubt concerning this point. If so, the source of heat is removed, the barium sulphate is allowed to settle for a short time, and a few drops of the reagent are caused to flow in: if this occasions further turbidity, more solution of barium chloride must be added. When the precipitation is completed, the beaker must be covered, and allowed to stand for at least two hours. The precipitate then settles, and the supernatant liquid may be easily poured off clear through a small filter, the precipitate being then repeatedly extracted with boiling water, which is each time poured off through the filter as soon as the precipitate has again subsided.

As soon as the filtrate which runs off no longer gives a chlorine reaction (§ 175), the precipitate is placed upon the filter, exactly as directed in § 7.

The filter is then dried, a platinum crucible is placed upon a sheet of glazed paper, and the precipitate is cautiously transferred to the crucible. The filter is burnt separately upon a platinum wire, the ash is added to the precipitate, heated for ten minutes, and weighed, after being allowed to cool in the exsiccator. The total increase of weight of the crucible consists of barium sulphate + the ash of the filter.

Ba . . .	136·9	S . . .	32
S . . .	32	O_3 . . .	48
O_4 . . .	64		
	232·9		80

In 232·9 *grm.* barium sulphate there are contained 80 *grm.* SO_3.
One milligramme barium sulphate represents x SO_3.

$$232{\cdot}9 : 80 = 1 : x. \quad x = 0{\cdot}3434.$$

If 123 *mgrm.* barium sulphate have been found, 123 ×

0·3434 = 42·2 *mgrm.* SO_3 are present. If this quantity has been obtained from 500 or 250 *cc.* of water, it must be multiplied with two, or respectively with four, in order to find the quantity in 1 litre.

Titration of Sulphuric Acid, according to Wildenstein.

(This method has hitherto been little in use, but according to Tiemann-Gärtner it is almost as accurate as the gravimetric method.)

§ 177. *Principle.*—If barium chloride is added in excess to the water, it precipitates all the sulphuric acid ; the excess of barium chloride is precipitated with a yellowish white colour by means of potassium chromate. The liquid does not take a yellow colour until the precipitation is complete.

Standard Solutions.—Decinormal barium chloride. 12·185 dry crystalline barium chloride ($BaCl_2 + 2H_2O$) are dissolved and made up to 1 litre, as

$$\frac{136{\cdot}9 + 2 \times 35{\cdot}4 + 2 \times 16 + 4 \times 1}{2 \times 10} = 12{\cdot}185.$$

One cubic centimetre of the solution exactly corresponds to 4 *mgrm.*, *i.e.*, $\frac{32 + 3 \times 16}{2 \times 10}$ *mgrm.* sulphuric acid. We also require a decinormal solution of potassium ammonium chromate.

Potassium bichromate is obtained perfectly free from sulphuric acid by re-crystallisation, dried between blotting-paper, and of the salt thus prepared 7·370 *grm.* are weighed off, and dissolved in about 100 *cc.* of water. The solution is poured into a litre flask, and ammonia is added, drop by drop, until the orange-red colour has become a pure yellow, with the formation of neutral potassium chromate and neutral ammonium chromate,

$$K_2Cr_2O_7 + 2NH_3 + H_2O = K_2CrO_4 + (NH_4)_2CrO_4.$$

It is then made up to 1 litre.

Equal volumes of the solutions of chromate and of barium chloride should give a yellowish white precipitate and a colourless solution, in which sulphuric acid should neither produce a white precipitate of $BaSO_4$, nor silver nitrate a brown coloration by the formation of Ag_2CrO_4.

Execution.—Water is treated as it is directed for the determination of permanent hardness, § 192.[1]

Of the water so treated, 100 *cc.* are heated to ebullition in a small flask, having a mark at 150 *cc.* ; from 10 to 20 *cc.* of solution of barium chloride are added, according as the water is poor or rich in sulphuric acid, boiling for a few minutes, and adding solution of potassium

[1] If water has not been previously well boiled, the bicarbonates will convert barium chloride into barium carbonate.

The consumption of each cubic centimetre of solution of soda represents the presence of 1 *mgrm.* of free carbonic acid. This method also determines CO_2 in waters which are coloured red by rosolic acid.

QUANTITATIVE DETERMINATION OF TOTAL CARBONIC ACID. (See § 180.)

§ 179*a*. *Principle.*—The entire carbonic acid is precipitated from the water, the precipitate is decomposed with an acid, and the CO_2 expelled is absorbed in standard baryta-water. The bicarbonates of the alkaline earths are precipitated by barium hydroxide, but the alkaline carbonates by barium chloride.

Execution.—A large glass flask is filled at the spring, closed immediately very well, and cooled down to from 4° to 5°. About 200 *cc.* are then drawn off with a syphon into another flask holding about 250 *cc.* Its quantity is determined exactly by weighing. There are now added 50 *cc.* baryta-water (about 20 *grm.* $Ba(OH)_2 + 0{\cdot}2$ *grm.* $BaCl_2$) per litre, and a few *cc.* of solution of barium chloride solution; it is carefully closed, allowed to stand for twelve hours, and the clear liquid is rapidly filtered through a small filter without disturbing the sediment. When this operation is nearly completed the filter is returned to the flask, and the process is continued as follows:—

The flask is closed with a caoutchouc stopper having three perforations. The middle aperture receives a bulb funnel with a cock, and it is filled with sulphuric acid at about 10 per cent. Of the two others the former serves for the entrance of air; it is a narrow tube extending nearly to the bottom of the flask and fitted with a Geissler potash-bulb, so that the air may enter free from CO_2. The remaining tube is for the exit of the CO_2 evolved, and conveys it firstly through a very small empty flask for condensed water, then through a Geissler sulphuric acid apparatus, and next through two Pettenkofer baryta-tubes, introduced in succession, and each containing about 200 *cc.* in which the absorption takes place. The titration and calculation are effected as for air. As oxalic acid there is conveniently used a solution containing per litre 2·86 *grm.* oxalic acid, therefore 1 *cc.* = 1 *mgrm.* CO_2.

To the last baryta-tube there is attached an aspirator which draws through the apparatus a very gentle current of air, freed from CO_2, and sweeps out all the CO_2. The CO_2 is produced by the slow admission of sulphuric acid; when the escape of gas becomes slackened it is heated for thirty minutes by means of a Bunsen burner, ebullition, however, being avoided.

QUANTITATIVE DETERMINATION OF COMBINED CARBONIC ACID BY TITRATION (according to Lunge).

§ 179*b*. *Principle.*—If sulphuric acid is added to a carbonate or bicarbonate, along with an aqueous yellow solution of methyl orange (§ 26), metallic sulphate and free carbonic

acid are formed, and the pale yellow colour remains unchanged until the carbonates are completely decomposed and a trace of free sulphuric acid is present, when a red colour appears. The presence of other salts has no importance. This method determines only the firmly combined carbonic acid, a molecule of the acid salt consuming exactly as much acid as a molecule of the neutral salt:—

$$CaCO_3 + H_2SO_4 = CaSO_4 + CO_2 + H_2O$$
$$Ca(HCO_3)_2 + H_2SO_4 = CaSO_4 + 2CO_2 + 2H_2O$$

Execution.—We prepare a sulphuric acid which saturates exactly as much solution of potassa as a solution of oxalic acid (2·863 *grm.* oxalic acid per litre), of which 1 *cc.* = 1 *mgrm.* carbonic acid.

We add this acid to the water coloured a light yellow with methyl-orange until it becomes red. If 9 *cc.* have been consumed, the water contains $5 \times 9 = 45$ *mgrm.* perfectly combined CO_2 per litre. As the change of colour of methyl-orange from yellow to red is not quite sudden, the determination is much facilitated by colouring 400 *cc.* of water an equally faint yellow with methyl-orange, measuring off the one half for titration, and reserving the other half for a check-test. A change of colour in the specimen which is being titrated is recognised with especial ease by comparison with the colour of the check-specimen.

CONJOINT QUANTITATIVE DETERMINATION OF THE FREE AND SEMI-COMBINED CO_2.

§ 180. *Principle.*—Pettenkofer proposes the following method: If a known volume of a solution of barium hydroxide (baryta-water) is added to the water under examination, the free CO_2 and the CO_2 in the bicarbonates will be deposited as insoluble $BaCO_3 + CaCO_3$.

$$CO_2 + Ba(OH)_2 = BaCO_3 + H_2O;\ 1Ba(OH)_2 = 1 \text{ free } CO_2$$
$$Ca < \begin{matrix} CO_3H \\ CO_3H \end{matrix} + Ba(OH)_2 = CaCO_3 + BaCO_3 + 2H_2O;\ 1Ba(OH)_2 = 1 \text{ semi-combined } CO_2.$$

Each molecule of $Ba(OH)_2$, which is converted into CO_3Ba,

and falls in an insoluble state to the bottom, represents a molecule of CO_2 in the free or the semi-combined state. This quantity results from the decrease of the alkalinity of the baryta-water before and after its addition to the water.

We have also to convert the alkaline salts containing acids, by which $Ba(OH)_2$ would be precipitated, *i.e.*, the carbonates, sulphates, and phosphates, into indifferent alkaline chlorides and insoluble barium salts by the addition of $BaCl_2$.

If magnesium bicarbonate is present we have the following transposition:—

At first $Mg\begin{matrix}CO_3H\\CO_3H\end{matrix}+Ba(OH)_2=BaCO_3+MgCO_3+2H_2O$, as in calcium bicarbonate;

but then $MgCO_3+Ba(OH)_2=BaCO_3+Mg(OH_2)$.

The magnesium hydroxide is precipitated. In a molecule of magnesium bicarbonate, the semi-combined carbonic acid precipitates not only as in calcium, barium hydroxide, but the second fully combined molecule of CO_2 is also transposed in the same manner. Hence, 1 molecule of MgO exchanges 1 molecule of free or semi-combined CO_2, *i.e.*, as often as we have 40 *mgrm.* MgO, we find 44 *mgrm.* CO_2 in excess, or for 1 *mgrm.* MgO 1·1 *mgrm.* CO_2. Formerly the attempt was made to avoid the transformation according to the second equation above given by the addition of ammonium chloride. Trillich, who has recently studied this method closely, found that the precipitation of the magnesium cannot be entirely prevented. He therefore adds no ammonium chloride, and takes the influence of the magnesia into account according to the above exposition. His method is (*Zeit. f. angewand. Chemie*, 1889, p. 337):—

1. The quantity of magnesium in the water is determined gravimetrically.

2. 100 *cc.* of water are mixed in a glass capable of being closed with 5 *cc.* solution of barium chloride, 1 : 10 and 45 *cc.* of titrated baryta-water (15 to 20 *grm.* barium hydroxide + 0·2 *grm.* barium chloride per litre), well shaken, and allowed to stand for twelve hours.

3. Of the clear liquid (150 *cc.*), two portions, each of 50 *cc.*, are drawn off with a pipette without disturbing the sediment, and after the addition of phenolphthaleine each is titrated with hydrochloric acid, of

which 1 *cc.* = 1 *mgrm.* carbonic acid, *i.e.*, it saturates as much alkali as an oxalic acid containing 2·863 *grm.* per litre of water. Oxalic acid and sulphuric acid may also be used.

If 100 *cc.* of water contain 5·8 *mgrm.* magnesia, and if, *e.g.*, 45 *cc.* of baryta-water = 108 *cc.* hydrochloric acid, and if 50 *cc.* of the clear liquid drawn off with the pipette require 27·4 *cc.* of hydrochloric acid for neutralisation, 150 *cc.* would have used 82·2, and the proportion of carbonic acid would be, in accordance with the difference in strength, 108 − 82·2 *mgrm.*; but as 5·8 *mgrm.* magnesium oxide took the place of 5·8 × 1·1 *mgrm.* carbonic acid, there are contained 108 − 82·2 − 6·4 = 19·4 *mgrm.*, or in 1000, 194 *mgrm.* of free and semi-combined carbonic acid.

The method, according to Trillich, is also available for the simple determination of the total carbonic acid. We colour the 50 *cc.* of liquid (together with the precipitate), remaining in the settling glass with a few drops of methyl-orange, or of tincture of cochineal, and add to it hydrochloric acid of the above strength, until the methyl-orange is reddened or the cochineal has changed from a rose to a yellow. If for this purpose there are used, *e.g.*, 68 *cc.* of hydrochloric acid, then the proportion of carbonic acid in 100 *cc.* in milligrammes is: 68 − 27·4 − (1·1 × 5·8) = 34·2, or per litre a quantity ten times greater; 27·4 is subtracted, because 50 *cc.* of liquid float above the precipitate and 1·1 × 5·8, because the precipitate consists in part of magnesium hydroxide containing 5·8, *mgrm.* MgO.

If G is the quantity of the total CO_2, x that of the free + the semi-combined, $G - x = B$, the quantity of the combined CO_2. But as the combined and the semi-combined are present in equal quantities, $G - 2B = F$, the quantity of the free carbonic acid. In water containing no free CO_2, $2B = G$, so that in many cases the more circumstantial determination of G may be evaded by simply titrating the combined carbonic acid by Lunge's method.

Example.—In the Würzburg water supply the various methods of determining carbonic acid gave per litre:

Total CO_2 according to Trillich, 350 *mgrm.* (according to § 179*a*, 353 *mgrm.*).	
Free and semi-combined (Pettenkofer and Trillich)	194
Combined (Lunge)	155
	349

So that, on the assumption that the quantity of the semi-combined

= that of the combined, 194 – 155 = 39 *mgrm.* free CO_2. According to § 179, I find it 32 *mgrm.* The water has a strong alkaline reaction, in consequence of its large proportion of bicarbonates. Compare Trillich, *Die Münchener Hochquellenleitung. Hygienische Tagesfragen*, viii. Munich, 1890.

5. Nitric Acid (Nitrates).

OCCURRENCE.

§ 181. Many pure waters contain no nitric acid at all, others only little. Very considerable quantities may be taken up from polluted soils. Nitric acid is generally combined with calcium.

QUALITATIVE RECOGNITION.

A. Detection of nitric acid, if no nitrous acid is present (nitrous acid yields the reactions of nitric acid, in addition to those peculiarly its own). The preliminary test for nitrous acid is effected as in § 183.

1. With brucine. A few cubic centimetres of the water are evaporated in a small porcelain capsule; two drops of pure concentrated sulphuric acid and a granule of brucine of the size of a pin's head are added; 1 *mgrm.* of nitric acid in a litre of water may thus be indicated by a rose colour. Care must be taken not to mistake for red the yellowish and brownish colours sometimes produced by a little dust, in the absence of nitric acid.

2. With diphenylamine, $N(C_6H_5)_2H$. We dissolve a few granules of diphenylamine in about 5 *cc.* of pure concentrated sulphuric acid, and add a few drops of the water being examined. Traces of nitrates and nitrites are shown by a blue coloration. If the result is negative, the water is previously concentrated by evaporation. If more water than $\frac{1}{10}$ of the volume of the concentrated sulphuric acid is added to the latter, the test fails, as the blue colour is destroyed.

3. By reduction of the nitrates to nitrites, and detection of the latter by the starch-iodine method.

For carrying out this test the water is mixed in a test-

glass with a few drops of concentrated sulphuric acid and a very little zinc powder is added. If much zinc is taken the test miscarries, as the N_2O_5 is reduced to NH_3. The reaction is:

$$NO_3H + H_2SO_4 + Zn = NO_2H + ZnSO_4 + H_2O$$

The process is concluded as indicated for nitrous acid, § 183.

4. By oxidising a blue dilute solution of indigo, a yellow colour appearing in its stead. In order to liberate the nitric acid, and to bring the water to the required heat, from 10 to 20 *cc.* of water in a small beaker are mixed with twice its volume of pure concentrated sulphuric acid, and dilute solution of indigo (for the preparation of which see the quantitative determination) is allowed to flow in. If for a time the indigo solution is turned to a yellow colour, or if a blue colour appears on the addition of a large quantity of indigo, nitric acid is present—the more so the larger the quantity of indigo consumed.

B. If nitrous acid is simultaneously present, the methods 1, 2, and 4 are useless. Method 3 may, however, prove serviceable unless with very large quantity of nitrous acid.

We compare the blue colour of the zinc-iodide starch before and after the reduction of the nitric acid. The more striking the difference, the greater is the proportion of nitric acid.

It is much more accurate to destroy the nitrous acid by adding a few drops of sulphuric acid and a knife-point full of pure urea (free from nitric acid) to 100 *cc.* of water:

$$2\,NO_2H + CO(NH_2)_2 = CO_2 + 2\,N_2 + 3\,H_2O.$$

The largest quantities of nitrous acid which are ever met with in drinking-water disappear if the specimen is allowed to stand for one hour at the temperature of a living-room, whilst very small quantities of nitric acid, which are simultaneously present, seem not to be in the least affected. I have frequently used this method, which hitherto has not been published.

QUANTITATIVE DETERMINATION.

The good methods which we possess for the determination of nitrates in pure aqueous solutions are unfortunately not quite accurate if applied to water, which, in addition to nitrates, contains also nitrites and organic matter, &c. Whilst I must refer the reader to the clear, critical, and appropriate discussion of the most important methods in Tiemann-Gaertner, I restrict myself here to the following exposition.

In cases where the greatest accuracy is required, it is necessary to work according to the method of Schulze-Tiemann, which depends on the reduction of the nitric acid to nitric oxide and the measurement of the latter. The directions given in Tiemann-Gaertner must be exactly followed, and experience in difficult laboratory work is a preliminary condition for success. For scientific research this method is necessary, but for practical purposes the following method is sufficient:—

DETERMINATION OF NITRIC ACID BY TITRATION WITH SOLUTION OF INDIGO (Marx-Trommsdorf).

§ 182. *Principle.*—See Qualitative Detection.

Preparation of the Solutions.—About 3 *grm.* of commercial indigotine (indigo-paste) are ground up in a mortar with about 60 *grm.* of pure concentrated sulphuric acid. There is formed chiefly disulpho-indigotic acid, along with some monosulpho-indigotic acid. After the mixture has been allowed to stand for twenty-four hours the conversion is complete, and the solution is poured into four volumes of distilled water. The very concentrated solution thus obtained is filtered after the insoluble monosulpho-indigotic acid has been deposited, and it may then be kept for a long time in tightly-stoppered bottles. From this stock liquid solutions fit for practical use can be obtained by dilution. Of the solutions used for titration, from 6 to 8 *cc.* should be decolorised by 1 *mgrm.* N_2O_5. Such a solution is slightly transparent in a wide burette (12 to 15 *mm.*).

For standardising we make up a solution of which 25 *cc.* = 1 *mgrm.* N_2O_5 nitric anhydride (1 litre = 40 *mgrm.* N_2O_5), and as we cannot weigh off this quantity we make up a solution containing 0·0748 *grm.* potassium nitrate per litre.

In 2 KNO_3 there is 1 N_2O_5;

$$2\,KNO_3 = 2(39 + 14 + 48);\ N_2O_5 = (28 + 80).$$

Hence there is 2 × 101 parts by weight of potassium nitrate, 108 parts by weight of N_2O_5; in how much potassium nitrate are there 40 N_2O_5? $202 : 108 = x : 40$; $x = 74{\cdot}84$ *mgrm.*

It is advisable to prepare a solution of 100 times this strength, *i.e.*, 7·484 *grm.* dissolved in 1 litre, and each time before use to dilute 10 *cc.*, measured exactly, to 1000 *cc.* The dilute solution cannot be preserved for a long time, but the concentrated liquid keeps well.

In executing a titration it is essential, both in standardising the solution and in every determination, to proceed absolutely in the same manner, since the action of mass, inequalities of temperature, &c., may easily render the results worthless. We must therefore work each time exactly according to the following instructions:—

25 *cc.* of the solution of potassium nitrate are mixed rather rapidly with 50 *cc.* of concentrated sulphuric acid, free from nitrates, in a flask containing from 120 to 150 *cc.*, shaking the flask round. The temperature rises to about 120° or 125°, and the nitrate is decomposed into sulphate and free nitric acid. The solution of indigo is caused to flow into this hot solution, at first drop by drop, and then[1] by entire cubic centimetres. The contents of the flask appear at first of a pale yellow, then transitory greenish colorations present themselves where the indigo solution is being mixed: gradually the greenish colour (mixture of yellow and blue) gives way more and more slowly to a yellow on shaking, and finally there arises a pale bottle-green colour, which persists for some minutes on standing quietly. The operator notes

[1] At the beginning we must be cautious with the addition: if the first cubic centimetres are let flow in too rapidly, a blue colour can remain in presence of a not inconsiderable quantity of nitric acid. The rapidity of the decoloration increases in the course of the titration.

the number of cubic centimetres of the indigo solution which have been consumed, and begins at once a second titration in the same manner, adding more boldly the quantity of indigo solution used the first time (always except the very first cubic centimetres), whereby the process is completed more rapidly and at a higher temperature, whence in general rather more indigo is consumed.

If in determining the standard decidedly less than 8 *cc.* indigo have been used for 1 *mgrm.* N_2O_5, the solution of indigo must be diluted until this proportion is approximately reached.

Titration of a Specimen of Water.—In examining a water we proceed in exactly the same manner. If in the first titration decidedly more than 8 *cc.* of the solution, duly diluted, have been consumed, we dilute the water for a succeeding titration with distilled water until for 25 *cc.* we now use about 8 *cc.* If, *e.g.*, 16·2 *cc.* of indigo were used for the undiluted water, the titration of the water is to be repeated after dilution with an equal volume of distilled water; if 37 *cc.* were consumed we dilute with a fourfold quantity.

Computation of the Titration.—Suppose that for 25 *cc.* of solution of potassium nitrate there have been used 8·5 *cc.* of the indigo solution. Therefore 8·5 *cc.* of indigo = 1 *mgrm.* N_2O_5. Suppose that for 25 *cc.* of a sample of water there have been used 6·4 *cc.* of the same solution of indigo, therefore for 1000 *cc.* there would be required $6{\cdot}4 \times 40 = 256$ *cc.* of indigo solution, which corresponds to $\frac{256}{8{\cdot}5} = 30{\cdot}1$ *mgrm.* N_2O_5 per litre.

In waters containing large quantities of organic substances these bodies, as they are themselves oxidised, withdraw a part of the indigo from oxidation, and hence the proportion of N_2O_5 found is too low. In such cases the determination of nitric acid must be undertaken in the final product which remains in the flask after determining the organic matter according to Kubel-Tiemann (§ 197). For this purpose the contents of the flask are poured into a measuring-glass; the flask is well rinsed out with distilled water, and the volume of the united liquids is made up to 300 *cc.* Of this quantity 25 *cc.* are used. The result must be multiplied by 3, as the 100 *cc.* originally applied in determining the organic substances were diluted to 300.

In this method it must further be considered that nitrous acid also acts upon indigo, but 108 parts N_2O_5 yield 48 parts of oxygen for oxidation, whilst 76 parts N_2O_3 furnish only 16 parts, *i.e.*, 1 *mgrm.* N_2O_5 acts almost exactly twice as strongly as 1 *mgrm.* N_2O_3. When the proportion of nitrite is considerable for every milligramme nitrous acid present per litre, $\frac{1}{2}$ *mgrm.* must be deducted from the nitric acid as determined by the Marx-Trommsdorf process, or the nitrites must be previously removed by means of urea (§ 181).

6. Nitrous Acid (Nitrites).

OCCURRENCE.

§ 183. Occurs only in polluted waters, and always in a relatively small proportion.

QUALITATIVE DETECTION.

Along with the reactions of nitric acid laid down in the foregoing section, nitrous acid always displays the following characteristic reactions:—

With Zinc-Iodide Starch.—Preparation of the reagent: 4 *grm.* starch are ground up with a little water to a milky paste, and then poured into 100 *cc.* boiling water containing 20 *grm.* zinc chloride. The mixture is then heated, whilst being constantly stirred until the starch is converted into paste. It is now diluted to 1 litre, 2 *grm.* zinc iodide are added, and the mixture is filtered in a closed closet, so as to be protected from light and preserved in a black bottle. 100 *cc.* of water are mixed with from 1 to 2 *cc.* of dilute sulphuric acid, and there are added about 3 *cc.* of the solution of zinc iodide starch. The iodine which is liberated turns the starch blue. In this manner a quantity of N_2O_3 in 1 litre so small as 0·02 *mgrm.* may be detected.

$$ZnI_2 + 2KNO_2 + 2H_2SO_4 = I_2 + ZnSO_4 + K_2SO_4 + 2NO + 2H_2O.$$

Tiemann-Gaertner affirm most decidedly that the presence of nitrates, ammonia, and organic matter does not in the least interfere either in the qualitative recognition or in the quantitative determination, and that the attempt to effect the reaction with acetic acid instead of sulphuric acid (as in

presence of sulphuric acid and organic matter the nitrates are reduced, and might produce a blue colour) is groundless. In presence of ferric compounds, which also liberate iodine, the method cannot be used. Heat renders the blue starch iodide colourless, but on cooling the colour returns. (See § 132 on substances which give or interfere with the starch-potassium iodide reaction.)

QUANTITATIVE REACTION. (Trommsdorf.)

Principle.—The process is effected colorimetrically according to the principles explained in § 28. If water contains from 0·1 to 0·4 *mgrm.* per litre N_2O_3, the method is directly applicable; if the proportion is greater, the water must be diluted accordingly. It must be remembered that the reaction of the water can be compared with a solution containing a known quantity of nitrite only if both are instituted parallel and quite simultaneously. The intensity of the colour increases for a considerable time.

Preparation of the Solution.—Since sodium nitrite is to be bought everywhere containing about 99 per cent. pure $NaNO_2$, and only about 1 per cent. of impurities, it is no longer necessary to prepare it personally, as for practical purposes this error does not come into consideration. If we dissolve 1·815 *grm.* of the purest sodium nitrite (which has previously dried over sulphuric acid in the exsiccator) in 1 litre, 1 *cc.* of the solution will exactly contain 1 *mgrm.* nitrous anhydride N_2O_3.

$NaNO_2$		N_2O_3	
Na	= 23	N_2	= 28
N	= 14	O_3	= 48
O_2	= 32		
	69		76

In two molecules of $NaNO_2$ there is contained one molecule of N_2O_3, or in 2·69 *grm.* sodium nitrite 76 *grm.* N_2O_3.

$$2 \times 69 : 76 = x : 1000 \text{ mgrm.} \quad x = 1815 \text{ mgrm.} = 1{\cdot}815 \text{ grm.}$$

10 *cc.* of the solution are diluted each time to 1 litre before use in order to obtain the practically useful degree of concentration, 1 *cc.* = 0·01 *mgrm.*, or 100 *cc.* = 1 *mgrm.* N_2O_3.

1, 2, 3, 4 *cc.* of this solution are poured into some upright cylinders of equal height and width, and each is filled up to 100 *cc.* with distilled water. The specimens of water to be examined are poured into similar cylinders; to each then is added 1 *cc.* of sulphuric acid at 30 per cent., and 2 *cc.* of the zinc-iodide starch solution (see foregoing page). It is convenient to have a specimen of distilled water alone without any addition of nitrite. One cylinder after the other (beginning with the weakest) is then closed with the palm of the clean hand and shaken

round. The cylinder with the largest proportion turns blue first, but we do not read off until the blueing of the water containing 1 *cc.* no longer increases after being left for five minutes. If the blueness in the water under examination is fainter than one, fresh specimens must be made up with 0·2, 0·4, 0·6, 0·8. If the intensity of the reaction intervenes between two and three, a set of specimens must be made up with 2·2, 2·4, 2·6, 2·8. If the reaction is stronger than that with 4 *cc.* of the nitrite solution, the water must be diluted with water free from nitrite and tested again; a fiftyfold dilution may be necessary in rare cases. Care must always be taken that the colours to be compared are produced simultaneously.

QUALITATIVE AND QUANTITATIVE METHOD WITH METAPHENYLENEDIAMINE (DIAMIDOBENZOL).

(Preusse and Tiemann.)

QUALITATIVE RECOGNITION.

§ 184. We dissolve 5 *grm.* pure metaphenylenediamine in a little water, add dilute sulphuric acid until the reaction is distinctly acid, and make up with distilled water to 1 litre. Of this liquid we add 1 *cc.*, and 1 *cc.* of sulphuric acid at 30 per cent. to 100 *cc.* of water. A yellowish brown or dark brown colour (triamidoazo-benzol = Bismarck brown) indicates nitrous acid:

$$2\,C_6H_4(NH_2)_2 + NO_2H = NH_2 . C_6H_4 . N : N . C_6H_3(NH_2)_2 + 2\,H_2O.$$

QUANTITATIVE DETERMINATION.

This is effected exactly as directed for the zinc-iodide starch method, with the aid of a colorimetrical comparison. 0·03 to 0·3 *mgrm.* per litre may thus be detected without dilution, but for larger quantities dilution is necessary. The solutions must be used so much diluted that distinct reaction appears only after one or two minutes. The observation continues for about twenty minutes. In coloured waters the method is not applicable, but they may be decolorised by adding to 200 *cc.* of water 3 *cc.* of solution of sodium carbonate (1 : 3) and 1 *cc.* of caustic soda-lye (1 : 4). The precipitated carbonates of the alkaline earths carry the colouring-matters down with them, and the further examinations are made in the clear supernatant liquid. To soft waters a couple of drops of solution of alum are first added. Organic substances, nitrates, and also small quantities of ferric salts, do not interfere if the water is acidulated with sulphuric acid in the manner described.

Large proportions of nitrites may be determined in the cold by titration with permanganate (see p. 351).

7. Phosphoric Acid (Phosphates).

OCCURRENCE.

§ 185. Absent in most pure waters, as phosphoric acid is very eagerly retained by the soil. Common in waste waters, sewage, &c.

If a precipitate is produced on exposing from 1 to 200 *cc.* of the water to prolonged ebullition, it contains all the phosphoric acid; it is filtered off, dissolved in a little nitric acid, and evaporated to dryness in a porcelain capsule. If no decided turbidity appears when a specimen of water is boiled, about 100 *cc.* are evaporated at once to dryness with a little nitric acid. The residue is heated for some time slightly above 100° (cautiously moving the capsule to and fro in a Bunsen flame), in order to render the silica insoluble. As soon as the capsule is cool the contents are dissolved in a little dilute nitric acid and filtered. The clear filtrate is added in a test-glass to about double its volume of a warm, clear (filtered previously if necessary), strong solution of ammonium molybdate in nitric acid,[1] and heated to about 60°, constantly trying the temperature of the glass with the hand, after holding it for a short time in the flame. Even very slight traces of phosphoric acid betray themselves by an incipient yellow coloration, which becomes more intense if the glass is set aside for a short time. Gradually (but rapidly, if the quantity of phosphoric acid is considerable) the clear liquid becomes turbid, and there is deposited a yellow precipitate of ammonium phospho-molybdate [2 $(NH_4)_3PO_4$ $(NH_4)_2$ HPO_4 36 MoO_3], whilst the supernatant liquid remains colourless.

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[1] 40 *grm.* ammonium molybdate are dissolved in 160 *cc.* of 10 per cent. ammonia (sp. gr. 0·96 at 14°), and 240 *cc.* water are added. The cold liquid is poured into 600 *cc.* of nitric acid at 27½ per cent. (sp. gr. 1·82). After standing for a few days it is filtered, and the solution is preserved in a dark closet.

QUANTITATIVE DETERMINATION.

Determination as Ammonium Phospho-molybdate.—From 1 to 4 litres of water are evaporated to dryness with nitric acid. In order to expel chlorides, to render silica insoluble, and to destroy organic substances, the residue is twice evaporated to dryness, each time with 50 *cc.* nitric acid. Finally the residue is dissolved in 10 *cc.* of dilute nitric acid, precipitated with 40 *cc.* of the above molybdic solution; $12\frac{1}{2}$ *grm.* crystalline ammonium nitrate are added, and the whole is allowed to stand in heat for twelve hours.

The precipitate is filtered off, washed out with a 20 per cent. solution of ammonium nitrate, which at first is slightly acidulated with nitric acid. When a drop of the washings no longer leaves a residue on the ignited cover of a platinum crucible, the washing is complete.

It is then once covered with distilled water, in order to remove the chief bulk of the ammonium nitrate, and the remainder is rinsed from the filter into a porcelain crucible. The residues of the precipitate which adhere to the paper are dissolved in a little dilute ammonia, the solution is concentrated, precipitated with nitric acid, and added to the contents of the crucible. The moisture is then expelled by evaporation on an asbestos plate, and the ammonium nitrate by very cautious heating over a double wire gauze. As soon as a watch-glass held above the crucible is no longer clouded (by the sublimation of ammonium nitrate), the crucible is placed in the exsiccator, and weighed when cold. The precipitate contains 3·8 per cent. P_2O_5.

If from 2000 *cc.* of water we have weighed 20 *mgrm.* precipitate, 1 litre contains: $\frac{20 \times 3{\cdot}8}{2 \times 100}$ *mgrm.* = 0·38 *mgrm.* P_2O_5.

This method is not very accurate, since the precipitate is not always uniform in its composition: according to Gaertner-Tiemann, however, it is the most suitable method for this purpose, on account of the small quantity of phosphoric acid present in water.

At the same time the best method of determining phosphoric acid may be mentioned, which must always be used in case of large quantities. The volumetric method, though often in use, gives only approximate results.

Determination as Magnesium Pyrophosphate.—We proceed exactly as described, but instead of weighing and drying the molybdic precipitate after being once washed with ammonium nitrate, it is dissolved, whilst still moist, in ammonia, the clear solution is mixed with 10 *cc.* of magnesia mixture[1] and one-third of the total volume of 10 per cent. liquid ammonia, and allowed to stand for from twelve to twenty-four hours, until the precipitate (ammonium magnesium phosphate) has been deposited in crystals.

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[1] Magnesia mixture: 50 *grm.* magnesium chloride + 70 *grm.* ammonium chloride + 350 *cc.* liquid ammonia at 10 per cent. (sp. gr. 0·96) + 750 water. Set aside for some days, and filter.

It is filtered off, and treated further as directed for the determination of magnesium (§ 190); the magnesium pyrophosphate, if multiplied by 0·639, gives the P_2O_5.

8. Hydrogen Sulphide, Sulphuretted Hydrogen, Sulphides.

OCCURRENCE.

§ 186. Found free in waters in which organic substances are putrefying, generally in combination with alkalies and alkaline earths; also in industrial waste waters, in which it is produced (§ 88) by Beggiatoa and other sulphur bacteria by the reduction of sulphates.

QUALITATIVE DETECTION.

1. On the addition of a solution of lead acetate or of copper sulphate there appears a yellowish brown or blackish coloration, and if the sulphur is abundant there is gradually deposited a black precipitate of lead or copper sulphide:

$$Pb(C_2H_3O_2)_2 + H_2S = PbS + 2\,C_2H_4O_2.$$
$$CuSO_4 + H_2S = CuS + H_2SO_4.$$

2. On the addition of soda-lye and some solution of sodium nitro-prusside there occurs a violet coloration if traces of sulphuretted hydrogen are present.

It is best first to precipitate the alkaline earths as directed for the determination of ammonia. (See § 194.)

QUANTITATIVE DETERMINATION.

We employ a colorimetric method with sodium nitro-prusside. For comparison we use sulphuretted hydrogen water of a known strength. It is obtained by passing a current of sulphuretted hydrogen gas into distilled water, which has previously been boiled and cooled. The gas is obtained from fragments of iron sulphide heated in a flask with dilute sulphuric acid. The sulphuretted hydrogen water is preserved in small bottles, each quite full, and carefully stoppered and kept in a dark closet. The proportion of

H_2S, which continually decreases, is ascertained as follows: 20 *cc.* are allowed to flow rapidly into 10 *cc.* of decinormal solution of iodine, and decinormal solution of sodium hyposulphite is added until the colour disappears. Each cubic centimetre which we use *less* than would be required for the titration of 10 *cc.* of the solution of iodine alone, corresponds to 1·7 *mgrm.* sulphuretted hydrogen (for details see § 135). The sulphuretted hydrogen water must be titrated afresh each time before it is used, and a fresh supply must frequently be prepared.

Execution of the Analysis.—From 300 *cc.* of the water under examination the alkaline earths are separated, as for the determination of ammonia (see § 194), and 250 *cc.* are then drawn off into a suitable cylindrical glass by means of a pipette, with the addition of 1 *cc.* of solution of sodium nitro-prusside (4 *grm.* to 1000 *cc.*). The colour is then compared, according to the principles of colorimetric analysis, with that of a set of specimens, each of 250 *cc.* distilled water, placed in cylinders exactly equal in size, with the addition of small measured quantities of the standard sulphuretted hydrogen water, 1 *cc.* of soda-lye, and 1 *cc.* of solution of sodium nitro-prusside. If the proportion of hydrogen sulphide is large, the water may be at once titrated with iodine and sodium hyposulphite, as directed above for the titration of the comparative solutions.

9. The Alkaline Earths. Calcium and Magnesium.

OCCURRENCE.

§ 187. These two nearly allied metals are scarcely absent in any water, and are preferably considered conjointly, since magnesium cannot be determined without the previous removal of the calcium. Both are further derived from the soil, and both are often determined conjointly by approximative methods (titration with soap). Both metals occur in water as bicarbonates, sulphates, and chlorides, and calcium is also met with in combination with nitric acid.

QUALITATIVE DETECTION.

Calcium.—If water is mixed with ammonium chloride and oxalate in abundance, and if a few drops of ammonia are added, the lime is precipitated as calcium oxalate. The ammonium chloride holds the magnesium oxalate in solution as a soluble ammonium magnesium oxalate is formed.

$$Ca\begin{matrix}CO_3H\\CO_3H\end{matrix} + \begin{matrix}COONH_4\\COONH_4\end{matrix} = \begin{matrix}COO\\COO\end{matrix}Ca + 2(NH_4)HCO_3$$

The quantity of the lime may be judged from the intensity of the turbidity.

Magnesium.—The filtrate from the lime precipitate which contains the magnesium as ammonium-magnesium oxalate is mixed with a solution of sodium phosphate and ammonia and stirred. There is then formed a precipitate of ammonium-magnesium phosphate, especially on rubbing the sides with a glass rod.

$$\begin{matrix}COO-NH_4\\COO\\COO\\COO-NH_4\end{matrix}>Mg + Na_2HPO_4 + NH_3 = 2\begin{matrix}COO-Na\\COO-Na\end{matrix} + MgNH_4PO_4$$

QUANTITATIVE DETERMINATION OF CALCIUM.

1. Gravimetric.

§ 188. We evaporate 500 *cc.* of hard water or 1000 *cc.* of soft water (slightly acidulated with hydrochloric acid) down to about 150 *cc.*, add solution of ammonium chloride, rinse it into a beaker glass having thin sides, and add finally NH_3 in excess. If a precipitate appears it consists of ferric hydroxide, aluminium hydroxide, and silica. If it is appreciable in quantity it may be filtered off, dried, ignited, and weighed. The ammonium chloride prevents the precipitation of $Mg(OH)_2$. After the liquid has been filtered into a second beaker (having thin sides) and the filter washed, the filtrate is boiled, a dilute solution of ammonium oxalate is added presently, and it is allowed to cool. It is then made up to 250 *cc.*, the precipitate of calcium oxalate is allowed to deposit and filtered through a small filter, which has previously been moistened with a little of the clear liquid. The first 200 *cc.* of the

filtrate are reserved for the determination of the magnesium, the precipitate is then brought upon the filter and washed with hot water until 3 *cc.* of the filtrate leave no residue if evaporated to dryness upon a platinum cover. The dried filter is placed in the platinum crucible over a Bunsen burner for incineration, then ignited upon the blast for conversion into CaO until the weight remains constant. If a blast is not at hand, the precipitate is collected upon a weighed filter, dried at 100° and weighed; it consists of $C_2O_4Ca + 2H_2O$, and contains 34·1 per cent. CaO.

If the proportion of magnesium is large it is necessary to redissolve the calcium precipitate in HCl, and again to precipitate with NH_3 and ammonium oxalate, as it contains magnesium oxalate. In this case all the filtrates are to be collected and to be concentrated to 200 *cc.* for the determination of the magnesium.

2. By Titration (Mohr's Method).

§ 189. By a circuitous process we may determine the lime volumetrically in a very practicable manner, and with sufficient accuracy for most hygienic purposes.

The principle of the method is: a quantity of water is mixed with an excess of oxalic acid and ammonia; a part of the oxalic acid is precipitated by the total lime; the portion of oxalic acid remaining in solution is determined by titration with permanganate, the precipitated portion is ascertained by subtraction, and from it the quantity of calcium is deduced.

Preparation of the Standard Solutions.—We make up a solution of oxalic acid, 25 *cc.* of which = 0·020 *grm.* CaO, or 1 litre = 0·8 *grm.* CaO, or 1 *cc.* = 0·0008 *grm.* CaO.

One molecule, *i.e.*, 56 *grm.* CaO, requires one molecule, *i.e.*, 126 *grm.* oxalic acid for precipitation, how much will 0·8 *grm.* CaO require?

$$0{\cdot}8 : x = 56 : 126.$$

$x = 1{\cdot}9$ *grm.* oxalic acid must be weighed out and dissolved in 1 litre of water. We then make up a solution of permanganate, of which about 10 *cc.* are required to oxidise 10 *cc.* of the solution of oxalic acid just mentioned.

Determination of the Standard.—A capsule is cleaned, as described below for the determination of the organic matter; into it are poured 100 *cc.* of water and 15 *cc.* concentrated

sulphuric acid, whereby the temperature is raised to about 60°; it is stirred with a thermometer, and, if needful, heated occasionally by means of a small flame.

The liquid is now coloured a faint rose by means of a few drops of potassium permanganate, for which very little is required, since distilled water contains no appreciable quantity of substances which act upon the permanganate at 60°. The permanganate burette is then read off, about 6 *cc.* of permanganate are added to the solution, and then 10 *cc.* oxalic acid, which destroys the colour, and permanganate is again added, until the original faint rose colour appears again and remains for some time.

If 10·3 *cc.* permanganate have been consumed, then 1 *cc.* permanganate = 0·97 *cc.* oxalic acid = x *mgrm.* CaO.

25 *cc.* oxalic acid = 20 *mgrm.* CaO; 0·97 *cc.* oxalic acid = x CaO; $25 : 20 = 0{\cdot}97 : x$; $x = 0{\cdot}776$ *mgrm.* CaO.

Practical Execution.—We pour into a small flask 300 *cc.* of distilled water, and mark its level with a writing diamond. The flask is then emptied, 100 *cc.* of the water to be examined are poured in, and so much liquid ammonia that the contents of the flask smell of it strongly. The flask is then heated to ebullition upon an asbestos plate, when the lime is deposited as calcium oxalate. The contents of the flask are then made up with water to the mark 300; it is well shaken round, and filtered from the sediment through a compact filter. When the filtration is nearly completed it is interrupted, 100 *cc.* of the filtrate are measured off, poured into a clean capsule, 15 *cc.* of concentrated sulphuric acid are added, and care is taken, as above, that the temperature remains constant at about 60°; permanganate is then caused to run in until a faint rose colour becomes permanent.

In our case we may suppose that 4 *cc.* of permanganate have been consumed for this purpose.

The calculation is best shown by an example: As 100 *cc.* water have been diluted to 300, and only 100 have been used for the analysis, the result obtained is to be multiplied by 3; 3 × 4 *cc.* = 12 *cc.* permanganate have been used in our case.

These 12 *cc.* of permanganate solution have oxidised 12 ×

0·97 *cc.* = 11·64 *cc.* of solution of oxalic acid. Of the 25 *cc.* of solution of oxalic acid added, 11·64 remained in solution, not having been precipitated by the lime, therefore 13·36 *cc.* have been combined with lime.

13·36 *cc.* oxalic acid represent 13·36 × 0·0008 *mgrm.* = 10·688 *mgrm.* CaO in 100 *cc.*, therefore 106·88 *mgrm.* = 0·1069 *grm.* CaO per litre.

QUANTITATIVE DETERMINATION OF MAGNESIUM.

§ 190. The 200 *cc.* of the filtrate from the determination of calcium which have been set aside (§ 183) are mixed with from 80 to 100 *cc.* of ammonia, and, if needful, with some ammonium chloride, in order to prevent a precipitation of $Mg(OH_2)$, and about 20 *cc.* of a saturated solution of sodium phosphate, mixed by stirring, without touching the sides of the glass (as adherent crystals are otherwise formed), and allowed to stand covered for twelve hours without the application of heat. The precipitate is collected upon a small filter, washed with dilute ammonia (one volume ammonia at specific gravity 0·96 to three volumes water) until 3 *cc.* of the filtrate leave on evaporation upon sheet platinum only the slightest dimness.

The chief mass of the precipitate is removed from the dried filter to a porcelain crucible, the filter is burnt on a platinum wire, the ash is allowed to fall into the crucible, heated first gently with the lid in its place, and then strongly with free access of air on a Bunsen burner and weighed. The weight of the magnesium pyrophosphate obtained $Mg_2P_2O_7$, if multiplied by 0·3603, gives magnesium oxide. As we have used not 250 *cc.* of the filtrate containing magnesium, but only 200 *cc.*, the result must be multiplied by $\frac{5}{4}$, in order to obtain the magnesium oxide in the quantity of water (500 or 1000 *cc.*) measured off for the determination of calcium.

An indirect determination of magnesium may often be sufficient.

From the total hardness subtract the quantity corresponding to CaO, and recalculate the remainder into magnesium oxide by multiplying by $\frac{40}{56}$. (Compare § 191.)

If the total hardness amounts to 31·5 German degrees, and if the water contains 270 *mgrm.* CaO, there are per litre $(315 - 270 = 45) \times \frac{40}{56} = 32{\cdot}1$ *mgrm.* referable to MgO.

SIMULTANEOUS DETERMINATION OF CALCIUM AND MAGNESIUM.

Clark's Determination of Hardness.

§ 191. Water which contains considerable quantities of the two alkaline earths above-named can be recognised by many persons by feeling with the hands as harder or less soft than distilled water. Leguminous vegetables cannot be boiled tender in it. On its evaporation there is deposited a quantity of a solid residue. Such water is therefore called *hard* in contradistinction to *soft* water, which is poor in the alkaline earths.

As the technical importance of hardness is very great, an attempt has been made to ascertain quantitatively the degree of hardness with rapidity and to record it, but unfortunately a different scale has been adopted in different countries.

Water has	As often as it contains
1 German degree of hardness.	1 *grm.* CaO in 100,000 *grm.*
1 French do.	1 *grm.* $CaCO_3$ in 100,000 *grm.*
1 English do.	1 *grm.* $CaCO_3$ in 70,000 *grm.*, *i.e.*, 1 grain (0·0648 *grm.*) in 1 gallon (4·543 litres).

1 French degree of hardness = 0·4 German degree.
1 English degree of hardness = 0·57 German degree.

In the following instructions German degrees are always understood. If water merely contains calcium carbonate, and if it has been determined gravimetrically or volumetrically, we merely need to divide by ten the number of the *mgrm.* CaO in 1 litre (= 1 million milligrammes) in order to find the German degrees of hardness. The water titrated above, containing 106·9 *mgrm.* CaO per litre, has 10·7 German degrees of hardness, if magnesium is not simultaneously present.

If both calcium and magnesium are present the latter is to be determined and recalculated as calcium, *i.e.*, we inquire how much CaO would be able to seize as much acid (to decompose soap), as 1 *grm.* MgO?

Ca = 40	Mg = 24
O = 16	O = 16
56	40

Forty parts MgO are equivalent to 56 CaO, or 1 *mgrm.* MgO is 56/40 = 1·4 *mgrm.* CaO.

The milligrammes MgO found in 1 litre are therefore to be multiplied by 1·4, and divided by ten, and the figure obtained is added to the proportion of CaO inferred from the degrees of hardness.

Principle.—For a speedy view of the degree of hardness of water we have a method which depends on the circumstance that alkali soaps (compounds of the alkalies with the fatty acids), froth with water, even if present in only small quantities, whilst calcium and magnesium soaps (compounds of the alkaline earths with the fatty acids) form insoluble precipitates. Thereby 56 *grm.* CaO have exactly the same effect as 40 *grm.* MgO, but we always express the result as if merely CaO were present.

I give here the method most frequently used for titration with soap—that of Clark—which, according to Tiemann-Gaertner, is at least equal in value, if not superior, to its modifications.

Reagents. Solution of Barium Chloride.—For standardising we use, instead of a solution of a known quantity of calcium (Clark took 12 *mgrm.* CaO in 100 *cc.* water), an equivalent solution of barium chloride, *i.e.*, we dissolve 0·523 barium chloride ($BaCl_2 + 2H_2O$), a salt easily obtained pure and free from moisture, in 1 litre.

Ba	136·9	Ca	40
Cl_2	70·8	O	16
$2H_2O$	36		
	243·7	:	$56 = x : 120$; $x = 523$ *mgrm.*

This solution has twelve German degrees of hardness; 100 *cc.* of the solution contain a quantity of barium equivalent to 12 *mgrm.* CaO.

Solution of Soap.—We grind up 150 *grm.* of lead plaster (a compound of lead and fatty acid), previously melted on the water-bath, to a uniform paste with 40 *grm.* potassium carbonate. The compound of potassium with fatty acid thus obtained is extracted with strong alcohol, filtered after it has

been allowed to settle, the alcohol distilled off, and the soap is dried in the water-bath. 20 *grm.* of this soap are dissolved in 1 litre alcohol of 56 per cent. by volume (specific gravity, 0·921).

Standardising.—We pour 100 *cc.* of the above solution of barium chloride into a bottle fitted with a ground glass stopper, and capable of holding 200 *cc.*, and we add soap solution (preferably from a Gay-Lussac burette, § 24), shaking all the time energetically, giving vertical shocks. We continue the addition of soap until, on laying down the bottle, a foam of fine bubbles remains for at least five minutes as a delicate film without breaking. For this purpose we have to use less than 45 *cc.*, *e.g.*, 38. In this case we add to each 38 *cc.* of the soap liquid 7 *cc.* of alcohol at 56 per cent., thus, *e.g.*, to 900 *cc.* $\frac{900 \times 7}{38} = 26{\cdot}3 \times 7 = 184{\cdot}1$ *cc.*, and observe if it is now correct, *i.e.*, if in reality exactly 45 *cc.* of soap liquid are required to form froth upon 100 *cc.* of solution of barium chloride.

Execution of a Determination of Hardness.—If we have a sample of soft water (river or lake water, or water from geological formations poor in lime), we proceed exactly as when standardising. To 100 *cc.* of water in the bottle we add, firstly, 1 *cc.*, and then a few cubic centimetres of the solution, shake the bottle, lay it down horizontally, and observe, watch in hand, if in five minutes there appears a distinct break in the froth. If the froth begins to break up soon after the shaking, we are still far from the completion of the titration; if the film persists for 4½ minutes before it breaks, only a few tenths of a cubic centimetre are needed for completion. If we have to titrate hard water (spring water from calcareous strata) we dilute it with from two to five parts of distilled water, as titration is possible only on the supposition that the hardness does not exceed 12°. Hard waters, if titrated without dilution, are sometimes deceptive in consequence of their proportion of magnesium, and very soon simulate a completion of titration in consequence of the production of a "false" froth of coarse bubbles. If we observe this phenomenon we must at once take in hand a new and diluted sample.

The consumption of the solution of soap is not proportional to the hardness; soft waters consume relatively more soap, perhaps because at great dilution there are formed double compounds of alkaline earths and of alkalies, each in combination with fatty acids. Table VIII. renders it practicable to ascertain the degree of hardness from the consumption of soap by means of simple interpolation.

If we have, *e.g.*, diluted a water to double its volume, and have used 22·8 *cc.* of soap liquid to 100 *cc.*, then 22·6 *cc.* correspond to 5·5 degrees of hardness, 23·0 *cc.* to 5·6 degrees; we find, therefore, for 22·8 soap 5·55 degrees of hardness. The undiluted water has therefore a hardness of $2 \times 5{\cdot}55 = 11{\cdot}1$.

§ 192. In the hardness of water technical chemistry distinguishes between *total hardness*, *permanent* or *persistent hardness*, and *temporary hardness*.

The *total hardness* is occasioned by all the salts of calcium and magnesium dissolved in water. We have just been engaged with its determination.

By *permanent hardness* we understand that which is found in water after it has been boiled for half an hour (when the calcium and magnesium carbonates are eliminated in consequence of the decomposition of the bicarbonates); it is chiefly occasioned by the sulphates, chlorides, and nitrates. The boiling must take place in a flask of known size, and care must be taken to add frequently distilled water in place of that which evaporates, otherwise a portion of the calcium sulphate is simultaneously precipitated. When the water is cold the flask is filled exactly up to the mark.

It must not be forgotten that $CaCO_3$ is not absolutely insoluble in water free from CO_2; in 100 *grm.* water 3·5 $MgCaCO_3$ remain in solution, and increase the permanent hardness by two German degrees.

By *temporary hardness* we understand that portion of the total hardness which depends on the carbonates, therefore total hardness minus permanent hardness. The temporary hardness can be directly ascertained with great ease by titration with sulphuric acid with methyl-orange as an indicator. (See § 206.)

Each cubic centimetre of decinormal sulphuric acid consumed shows how many times 2·8 *mgrm.* CaO, or the equivalent quantity of magnesium, is present as bicarbonate.

Example.—100 *cc.* consumed 4·2 *cc.* decinormal sulphuric acid, consequently per litre 4·2 × 2·8 × 10 = 117·6 *mgrm.* CaO as bicarbonate = 11·8 German degrees of temporary hardness.[1]

10. Alkali Metals.

§ 193. An exact determination of the alkali metals requires a skilful chemist. We cannot enter upon the details of this very circumstantial analysis, as it is of secondary importance for hygienic purposes.

Principle.—We may merely give the general principles of the process. After the alkaline earths and the other metals have been removed by precipitation, a measured portion of the filtrate is evaporated, dried, and the sodium and potassium chlorides are weighed conjointly. The alkaline chlorides are now converted into double compounds with platinum chloride; potassium platinum chloride is insoluble in a mixture of alcohol and ether. Sodium platinum chloride passes into solution. From the weight of the dried potassium platinum chloride, or from that of the reduced metallic platinum the potassium is found, and the sodium by subtracting that of the potassium (or the potassium chloride) from the sum of the alkalies.

11. Ammonia.

OCCURRENCE.

§ 194. Ammonia is entirely absent in superior drinking waters; it is often produced by the vital action of the microbia in water, but is very readily absorbed by the soil.

QUALITATIVE DEMONSTRATION.

Nessler's reagent, a solution of $HgI_2 . 2KI$, a double compound of mercuric iodide and potassium iodide in potassa-

[1] See *Water Analysis*, by J. Alfred Wanklyn, 3rd edition, 1874, or any of the subsequent editions. Chapter VIII.: Hardness.—*Editor.*

lye[1] yields with ammonia an orange red precipitate of ammonium mercuric iodide.

$$NH_3 + 2HgI_2 . 2KI + 3KOH = \begin{matrix} & & H \\ & \diagup & H \\ N & = & Hg \\ & = & Hg \\ & \diagdown & I \end{matrix} > O + 7KI + 2H_2O.$$

If only very trifling quantities of ammonia are present in the solution, there appears not a precipitate but a yellow colour in the liquid by which the smallest traces may be detected with certainty (0·05 *mgrm.* in 1 litre). Care, however, must be taken that there is not formed a precipitate of calcium carbonate on the addition of the alkaline reagent

$$(2KOH + (CO_3H)_2Ca = CaCO_3 + K_2CO_3 + H_2O),$$

which carries down the fine suspended yellow particles, and greatly interferes with the sharpness of the reaction.

Before testing, we add, therefore, to 300 *cc.* of the water in a stoppered cylinder 1 *cc.* soda-lye (1 : 4), and 2 *cc.* sodium carbonate (1 : 3), and allow the cylinder to stand over night undisturbed until the precipitate has subsided. We then draw off with a pipette a portion of the clear supernatant liquid, add to it about 1 *cc.* of the Nessler reagent and observe the colour. The reagents must, of course, be free from ammonia. Soda-lye free from ammonia is obtained by preparing the solution 1 : 4, and heating until a quarter of the water is evaporated; pure sodium hydroxide dissolved in pure water can be freed from traces of ammonia by heating for a short time.

QUANTITATIVE DETERMINATION.

Principle.—On account of the very small quantity of ammonia found in drinking water, we are generally content with a colorimetric determination. We compare the intensity

[1] 50 *grm.* KI are dissolved in about 50 *cc.* of hot water, and precipitated with a hot concentrated solution of mercuric chloride until there appears a red precipitate of HgI_2, which does not disappear on agitation. After filtration we add 150 *grm.* KOH in 300 *cc.* of water, dilute to 1 litre, add 5 *cc.* solution of mercuric chloride, allow the liquid to settle, and decant off the clear solution. It is kept in well-stoppered bottles; if a sediment is gradually formed the supernatant liquid is drawn off for use with a pipette.

of the colour of our sample with a dilute solution of ammonium chloride after the addition to each of equal quantities of the Nessler reagent. The water to be examined must previously be freed, as described, from alkaline earths.

Preparation of the Standard Comparative Solution.—In order to obtain a solution which contains 1 *mgrm.* ammonia in 1 *cc.* we weigh out 3·141 *grm.* of ammonium chloride, which has been dried over sulphuric acid, and dissolve it to make up 1 litre.

N 14			N 14
H_4 4			H_3 3
Cl 35·4			
in 53·4 *grm.*	ammonium chloride there are		17 *grm.* ammonia.

$$53{\cdot}4 : 17 = x : 1 \qquad x = 3{\cdot}141.$$

Therefore 3·141 ammonium chloride = 1 *grm.* ammonia.

From this solution we make up before use one more dilute; 50 *cc.* diluted to 1 litre (*i.e.*, twentyfold) yield a solution 1 *cc.* = 0·05 *mgrm.* NH_3.

Of this dilute solution we add 0·2 to 1 *cc.* to each 100 *cc.* of water free from ammonia in cylinders of equal width, and then 1 *cc.* of Nessler reagent until we obtain a specimen of water equal in depth of colour to the specimen under examination. If the latter after five minutes gives a stronger reaction than the comparative specimen with 1·0 *cc.*, a new specimen of the water under examination (after being freed from the alkaline earths) must be correspondingly diluted with water free from ammonia (*e.g.*, to one half), and 100 *cc.* of this dilution are again treated with the Nessler reagent. A specimen which has been already mixed with the reagent must never be *subsequently* diluted with water; still less must more solution of ammonium chloride be *subsequently* added to a specimen. The coloured reactions of the comparative solution remain unaltered for some hours, and if several determinations have been made they may therefore serve as a comparison for all. However, we prefer to read

off after five minutes. It is necessary that the water and the comparative solution have equal temperatures.

It is often by no means easy to obtain distilled water free from ammonia. Recently distilled water, if the first portion of the distillate has been rejected, gives no reaction for ammonia; but if it has been kept in the laboratory, especially in open or badly stoppered vessels, it shows distinctly the reaction of ammouia (and frequently also those for nitric and nitrous acids). It is often, therefore, more convenient to make up the comparative specimens with clean tap water, which in most cities contains not a trace of NH_3. Here, however, care must be taken for a previous precipitation of the alkaline earths.

Quantities of ammonia, which are too minute to be indicated by the Nessler reagent, have no hygienic interest. Larger quantities of ammonia, such as occasionally occur in sewage and in industrial waste waters after suitable dilution, may be determined colorimetrically with sufficient accuracy. It is more accurate to distil off the ammonia, convert it into ammonium platinum chloride, to ignite, and to weigh the metallic platinum.

12. Iron.

OCCURRENCE.

§ 195. As traces in many waters, especially those of deep wells in the plains of North Germany, as ferrous carbonate. (See § 170.)

QUALITATIVE RECOGNITION.

We collect the precipitate formed on boiling water for a length of time (determination of permanent hardness) upon a small filter, wash the flask in which the water has been boiled with dilute hydrochloric acid, and throw the filter into it. The contents of the flask are filtered off and concentrated by evaporation, after adding a knife-point full of potassium chlorate. When it is cold we obtain, if iron is present, a blue colour with potassium ferrocyanide, and a red colour with potassium sulphocyanide. The solutions of potassium

ferrocyanide and sulphocyanide should often be prepared fresh, especially as the former, if it has been kept for a long time, often gives a blue colour with hydrochloric acid alone without the presence of iron.

QUANTITATIVE DETERMINATION.

This is effected colorimetrically. From 200 to 500 *cc.* of water are evaporated down to about 50 *cc.*, along with a small knife-point full of potassium chlorate and 1 *cc.* of concentrated hydrochloric acid, free from iron, in order to peroxidise any ferrous compounds which may be present, whilst any free Cl escapes. It is then made up to 100 *cc.* with distilled water, and this quantity is used for analysis if the proportion of iron is small. If it is large a portion only is taken and made up to 100 *cc.*

A comparative solution is obtained by dissolving 0·898 *grm.* pale violet iron alum ($Fe_2(SO_4)_3 + K_2SO_4 + 24H_2O$) in 1 litre water. The iron alum is previously carefully dried with blotting-paper; 1 *cc.* of the liquid contains 0·1 *mgrm.* of iron. Of this solution we place respectively 1, 2, 3, 4 *cc.* in suitable cylinders filled up to 100 *cc.* To each of these four, and to the water to be examined, we add 1 *cc.* of a pale solution of potassium ferrocyanide, and ½ *cc.* hydrochloric acid. An accurate determination of the intensity of the colour is practicable only with quantities of 1 to 5 *mgrm.* per litre; with smaller proportions the colour is too faint, and with larger proportions too deep.

13. The other Heavy Metals (Lead, Copper, Zinc, Tin).

§ 196. These substances occur in water almost exclusively when it has remained in vessels, pipes, &c., of these materials. Their detection and determination are effected exactly according to the directions laid down in § 462. In order to obtain these substances, which are generally present only in traces, in a form suitable for analysis, we evaporate 1 litre, after acidulation with hydrochloric acid, down to about 200 *cc.*

14. Organic Substances.

OCCURRENCE.

§ 197. Along with the inorganic constituents, which are generally well known, most waters contain larger or smaller quantities of organic substances, which as yet are but little known. There have been hitherto found: humic substances (see soils), fatty acids (formic, acetic, propionic, and butyric acids), nitrogenous basic compounds, hydrocarbons, &c. The separate determination is either impracticable, or requires the attention of a chemist working independently.

QUANTITATIVE DETERMINATION (according to Kubel-Tiemann).

Principle.—The hygienist must hitherto be content with an approximate insight into the quantity of organic matter by means of very rough methods. It is shown above (§ 174) on what grounds the determination of organic substances from the loss or ignition of the evaporated residue—formerly so much in vogue—has been entirely abandoned. But, unfortunately, we hitherto possess in its stead no method which is perfectly satisfactory.

Still, among the numerous methods which have been proposed, one which is easy of execution has come widely into use, and, in default of a better, deserves to be further employed, especially as it affords us at least certain data. This method, which in the form here described has been worked out by Kubel-Tiemann, depends on the idea of determining how much nascent oxygen the organic substances of a specimen of water are able to take up; the more oxygen is consumed for the oxidation of these substances the larger is the proportion of organic substances supposed to be present.

Even simple reflection tells us that, *e.g.*, 1 *grm.* sugar and 1 *grm.* fat require very different quantities of oxygen for their oxidation, whence this method cannot possibly tell us the weight of the organic matter. In the second place it is manifest that different organic substances will be oxidised with unequal facility, that probably substances not easily

oxidised will remain unattacked, whilst others readily oxidisable will be totally destroyed. Further, inorganic bodies capable of higher oxidation, *e.g.*, nitrous acid, ferrous oxide, can also take up oxygen, as they are converted into nitric acid, ferric oxide, &c. These *à priori* objections are, according Schulze, but too well justified.[1]

Preparation of the Standard Solutions.—As the source of oxygen we use potassium permanganate ($KMnO_4$). The reduction of the permanganate proceeds—of course only on condition of the presence of substances capable of oxidation, and of the simultaneous presence of sufficient sulphuric acid—as follows:—

$$2KMnO_4 + 3H_2SO_4 = 2MnSO_4 + K_2SO_4 + 3H_2O + 5O.$$

Therefore from two molecules of the violet potassium permanganate there are evolved two molecules of colourless manganese sulphate, and five atoms of free oxygen.

$$KMnO_4 = 39 + 54{\cdot}8 + 4 \times 16 = 157{\cdot}8.$$

Hence $2 \times 157{\cdot}8 = 315{\cdot}6$ *grm.* potassium permanganate, $5 \times 16 = 80$ *grm.* oxygen, or $\frac{315{\cdot}6}{80} = 3{\cdot}945$ *grm.* permanganate, yield 1 *grm.* oxygen, and 0·3945 *grm.* 0·1 *grm.* oxygen.

It would be easy to produce such a solution exactly if the permanganate were not always somewhat impure, so that we never know accurately how much permanganate is actually present in a weighed quantity. Further, the solutions are not permanent, and must be standardised anew whenever

[1]

	By Tartaric Acid.	By Glucose.	By Cane Sugar.	By Benzoic Acid.	By Phenol.	By Leucine.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
On boiling for ten minutes there are consumed of the proportion of oxygen theoretically mixed . . .	95·6	61·0	55·1	3·7	73·5	11·4
On boiling for five minutes	75·0	42·7	53·8	2·1	41·1	10·8

they have to be used. We weigh out, therefore, about 0·4 *grm.* potassium permanganate, dissolve it in 1 litre water, and determine then the exact value of the solution as follows:—

Oxalic acid at the temperature of ebullition in the presence of permanganate is at once exactly split up into carbonic acid and water.

$$\begin{matrix}\text{COOH}\\|\\\text{COOH}\end{matrix} + O = 2CO_2 + H_2O.$$

As oxalic acid contains two molecules of crystalline water (see § 128), its molecular weight = 126; 126 *grm.* oxalic acid consume 16 *grm.* oxygen. We prepare, therefore, a solution of oxalic acid, of which 10 *cc.* exactly consume 1 *mgrm.* oxygen, and consequently a litre consumes 100 *mgrm.* = 0·1 *grm.*

$$126 : 16 = x : 0{\cdot}1.$$

$x = 12{\cdot}6 : 16 = 0{\cdot}7875$ *grm.* oxalic acid are to be dissolved in 1 litre.

We next determine how many cubic centimetres of solution of permanganate are decolorised by 10 *cc.* of this solution of oxalic acid; the number found shows how many cubic centimetres of solution of permanganate yield 1 *mgrm.* oxygen.

The Titration.

(*a.*) *Cleaning the Flask.*—Into an Erlenmeyer flask of the capacity of 300 *cc.* we pour from a Gay-Lussac burette a few cubic centimetres of the solution of permanganate, and also 5 *cc.* of 25 per cent. sulphuric acid, and about 50 *cc.* of distilled water. We then boil for five minutes upon a wire gauze, agitating the flask continually, and thus destroy any traces of organic matter adhering to the flask, and pour the liquid away.

(*b.*) *Standardising the Oxalic Acid.*—There are poured into the flask 100 *cc.* distilled water, and 5 *cc.* sulphuric acid at 25 per cent. It is heated to ebullition, and so much solution of permanganate is added that a permanent, distinct, but faint rose colour is obtained. It is boiled for five minutes, and

the addition of permanganate is continued according as the rose colour disappears. We thus obtain 100 *cc.* of water free from organic matter. We read off the burette, and add to this water 6 to 7 *cc.* of solution of permanganate, and 5 *cc.* of sulphuric acid at 25 per cent. We then boil, but not for five minutes, and add 10 *cc.* of oxalic acid, which occasions a destruction of colour, and add then permanganate until a faint permanent rose colour returns, when we again read off the burette. If the difference of the readings amounts, *e.g.*, to 9·5, then 9 5 *cc.* permanganate are capable of yielding 1 *mgrm.* oxygen; therefore 1 *cc.* of permanganate yields $\frac{1}{9 \cdot 5}$, *i.e.*, 0·105 *mgrm.* oxygen. Before every series of researches the standard of the solution of permanganate must be determined anew. The oxalic acid will keep from eight to fourteen days, but it is then destroyed by the growth of hyphomycetes.

(*c.*) *Performance of a Water Analysis.*—The Erlenmeyer flask is emptied; 100 *cc.* of the water to be examined are introduced, and 5 *cc.* of sulphuric acid at 25 per cent. are poured in, with a drop of solution of permanganate to obtain a rose colour. The permanganate burette is read off, 8 *cc.* are poured into the flask, and the mixture is boiled for ten minutes, counting from the first bubble.

If, after boiling, the colour becomes pale, some cubic centimetres of permanganate are again added; the flame is then removed, 10 *cc.* of oxalic acid are poured in, and solution of permanganate is added afresh until the colour is a faint rose. The burette is then again read off. If 13·5 *cc.* solution of permanganate have been consumed by the oxalate and the organic substance, there come to the account of the latter $13 \cdot 5 - 9 \cdot 5 = 4$ *cc.* One litre of water, therefore, consumes 40 *cc.* permanganate, and as 9·5 correspond to 1 *cc.* of oxygen, $\frac{40}{9 \cdot 5} = 4 \cdot 2$ oxygen.

If it appears on titration that the first 6 or 7 *cc.* of permanganate added are decolorised very rapidly, that is, if the water is very impure, it is well, in order to avoid errors, to use only 20 or 30 *cc.* of the water, diluting it with 80 or 70

cc. of distilled water, purified as in standardising the oxalic acid, and to determine the organic substance in the water thus diluted. If the water is not completely decolorised on addition of the oxalic acid, and if flocks or dark granules separate out, we add 5 *cc.* more of sulphuric acid.

Many authors boil only for five minutes, which is a decided economy of time, but, according to the above, less accurate.

If the water contains nitrous acid, this also has a reducing effect upon the potassium permanganate. This reaction takes place even at the temperature of a room, *i.e.*, at a temperature at which organic substances do not react appreciably.

$$NO_2H + O = NO_3H.$$

Hence 76 *grm.* N_2O_3($N_2 = 28$, $O_3 = 48$), 32 *grm.* oxygen; 1 *mgrm.* oxygen suffices for oxidising 2·377 *mgrm.* N_2O_3.

$$76 : 32 = x : 1$$
$$x = 2{\cdot}377 \text{ } mgrm.$$

For performing the titration we pour into a flask, cleaned as above, 100 *cc.* of the water in question, and 5 *cc.* of sulphuric acid at 25 per cent., and add whilst stirring, in the cold, the solution of potassium permanganate until a permanent rose colour makes its appearance.

If for this 0·8 *cc.* permanganate have been required, we calculate the proportion of N_2O_3 as follows: 9·5 *cc.* of our solution yield 1 *mgrm.* of oxygen, *i.e.*, are able to oxidise 2·377 *mgrm.* N_2O_3, therefore 0·8 *x mgrm.*

$$9{\cdot}5 : 2{\cdot}377 = 0{\cdot}8 : x.$$
$$x = 0{\cdot}20 \text{ } mgrm. \text{ } N_2O_3, \text{ } i.e., \text{ in 1 litre } 2{\cdot}0 \text{ } mgrm. \text{ } N_2O_3.$$

In order to use the result of the titration of the nitrous acid as a correction in determining the organic substances, we simply deduct from the quantity of permanganate consumed in determining the organic matter in 100 *cc.* of water, the quantity used on determining the nitrites in the cold, and then calculate from the difference the oxygen consumed by the organic substances.

Returning to our example:—

100 *cc.* permanganate consumed on boiling . . 4·0 *cc.* (for organic matter and nitrites);

100 *cc.* permanganate consumed in the cold { 0·8 *cc.* (nitrites alone); 3·2 *cc.* of solution of permanganate consumed by the organic substances alone.

$\frac{3{\cdot}2}{9{\cdot}5} = 0{\cdot}33$ *mgrm.* oxygen have therefore been consumed in the oxidation of the organic substances alone.

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The presence of ammonia does not interfere in the determination of organic matter.

The result of the determination of the organic substances is given :— The organic substances in 1 litre require for oxidation (in our example) 3·3 *mgrm.* oxygen or 3·3 × 3·94 = 13·03 *mgrm.* permanganate. Formerly the quantity of oxygen × 20, or the permanganate × 5 = organic matter, a perfectly arbitrary assumption, as permanganate × 3·43 in case of benzoic acid, and permanganate × 0·14 in case of phenol, would give correct values (a fluctuation of thirty times).

The more circumstantial titration of organic matter with permanganate, as proposed by Schulze, first in an alkaline, and then in an acid liquid, presents no advantages; the quantity of organic matter oxidised is only very slightly larger.

15. Substances Rarely Determined.

§ 197*a*. Excrements may be recognised, according to Fleck, by evaporating 2 litres of the water to dryness with 2 *grm.* tartaric acid, extracting the residue with alcohol, and evaporating off the alcohol. On the addition of potassa-lye to the alcoholic extract fæcal matter may be detected by the smell.

If urine is largely present uric acid may be recognised. The residue, after evaporation to dryness, is boiled with a little sodium carbonate, the sodium urate formed is filtered off, evaporated down in a small porcelain capsule, treated with nitric acid, and evaporated to dryness. The yellowish red colour which appears becomes purple red on the addition of ammonia, or blue on treatment with soda-lye. (Murexide reaction.)

Wolff, Degener, and Herzfeld have proposed a method for determining the carbon contained in water in the state of organic, non-volatile compounds (though about 10 per cent. of them is volatile). The method, according to Tiemann-Gaertner, is accurate, but it can be used only by experienced analysts. After all the pre-existing carbonic acid has been expelled, the organic carbon is converted by chromic acid into CO_2, which is weighed. As the proportion of carbon in the substances in question varies "only" between 30 and 80 per cent., the organic matter can be calculated rather more accurately from the carbon present than from the permanganate consumed. This method has proved useful for the examination of the waste waters from sugar-works, where the percentage of organic matter is large and its composition uniform.

For the determination of nitrogen it is preferable to use Kjeldahl's method (§§ 212 and 213). If the water does not contain more than 450 *mgrm.* nitric acid per litre, it is best to determine the nitric nitrogen simultaneously, converting it into ammonia prior to the evaporation of

the water by cautious heating with a little sulphuric acid, zinc chloride, and a drop of platinum chloride.

In England preference is given to the determination of the albumenoid ammonia, the NH_3 which is split off from organic substances on treatment with an alkaline solution of permanganate (Wanklyn, Chapman, and Smith).

As water rich in organic substances contains but little free oxygen, determinations of the absorbed oxygen have been undertaken. The gas expelled by boiling from a specimen of water (obtained with especial precautions) is collected over soda-lye, and determined according to the rules of gas analysis as oxygen and nitrogen, with the aid of hydrogen or pyrogallic acid. The method is practicable only for the trained chemist, and is very useful as a complementary method in the examination of very impure river waters, &c. More convenient is the method proposed by Schutzenberger and Risler. Here the quantity of sodium disulpholeukindigotate which can be converted into the corresponding blue salt by the oxygen present is determined.

A series of especial reagents are here required, the description of which cannot be undertaken. A litre of pure water, at a pressure of 760 *mm.*, can take up at 0° 8·63 *cc.*, and at 10° 6·81 *cc.* of oxygen. All the methods may be found fully described in Tiemann-Gaertner.

The best method seems to be that of Wilhelm Winkler. According to it there is added to the water manganic chloride and soda-lye. Manganic hydroxide is thus formed, which, in contact with potassium iodide and hydrochloric acid, is reconverted into manganic chloride setting free a quantity of iodine equivalent to the quantity of oxygen, and which can be easily determined by titration with centinormal sodium thiosulphate (*Berichte der Deutsch. Chem. Gesellsch*, 1888, p. 2843, 1889, p. 1764. See also Kisch, *Zeit. f. Anal. Chemie*, 1891, No. 4).

In water polluted by industrial drainage there may naturally occur very different impurities, which cannot here be considered separately. The most varied salts, arsenic, dye-wares, sugar, starch, and in gas water, sulpho-cyanides, and cyanides.[1]

16. Collocation of the Results.

§ 198. The results of the analysis are given by stating how many milligrammes have been found in 1 litre, or how many parts in 100,000 parts.[2] The latter expression represents milligrammes in 100 *cc.*, *a* parts in 100,000 parts = *a mgrm.*

[1] It is illegal in England to allow gas refuse to flow into any river, sewer, or canal.—*Editor.*

[2] In England the reports are given either as grains per gallon, or as parts in 100,000. The latter expression is preferable, as it is the more readily convertible into the metric system.—*Editor.*

in 100 *cc.* = 10 × *a mgrm.* in 1 litre; *b mgrm.* in 1 litre = $\frac{b}{10}$ *mgrm.* in 100 *cc.* = $\frac{b}{10}$ parts in 100,000.

As in 100 *cc.* of water the single constituents are present often in very minute traces, I have, in common with many recent works, taken the number of milligrammes per litre as the basis in this book.

In general, we are satisfied with stating the metals as oxides, the ammonia as such, and the acids as anhydrides. The recalculation into the salts really present in the water is only of limited hygienic interest. We generally proceed as follows:—

The chlorine present is assumed to be combined with sodium; if there is any chlorine over it is assigned to potassium, or, in default, to calcium. This, however, is rarely needful, as the chlorine and sodium usually correspond. If there is an excess of sodium, it and the potassium are allotted to the sulphuric acid, and any excess of this acid is assigned to calcium. We may infer the presence of considerable quantities of sodium or potassium sulphate if the quantity of calcium, which follows from the permanent hardness, is much smaller than is necessary to take up the sulphuric acid.

Nitric acid is assumed to be combined with the ammonia; the excess (often the main portion) is allotted in pure waters to lime, but in polluted waters to fixed organic bases. Calcium and magnesium, if not combined with sulphuric acid, and possibly to nitric acid or chlorine, are present as bicarbonates. We generally tabulate the carbonate, and along with it the semi-combined carbonic acid. Silica is given as SiO_2, iron and alumina as Fe_2O_3 and Al_2O_3, nitrous acid as N_2O_3, but they are not grouped as salts on account of their small quantities.

Tiemann-Gaertner has been led to assume the presence of organic bases in strongly polluted waters by the demonstration that in water, which has been boiled to determine permanent hardness (§ 191), the determination of hardness shows a decidedly lower proportion of lime that would be necessary to neutralise the portions of HCl, H_2SO_4, and NO_3H

not taken up by alkaline and ammonia. But as such waters always have a neutral reaction unknown organic bases, not to be expelled by ebullition, must aid in saturating the acids. (See Tiemann-Gaertner, p. 377.)

III. *MICROSCOPICAL EXAMINATION OF WATER.*

§ 199. If water is not quite clear, and if it deposits a sediment when left in a tall, conical glass for some hours, the deposit should be at once examined under the microscope with a magnifying power of 200 to 300 diameters. An enumeration of the extremely manifold foreign bodies occasionally met with in water would take us too far, hence they can only be mentioned in groups.

1. Dead objects belonging to all the kingdoms which have been carried into the water, but which do not give any direct evidence of pollution by men or other animals.
   - *a.* Grains of sand, particles of clay, suspended insoluble humic bodies.
   - *b.* Fragments from the vegetable kingdom: particles of wood and bark, portions of the tissues of herbaceous plants, especially straws and grains of pollen.
   - *c.* Bodies of animals, entire or in portions. Hairs, chitinous armour of insects and crustaceans, &c.

2. Objects which prove that the refuse of animal (especially human) economy have found their way into the water.
   - *a.* Fragments of textile fibres: cotton, linen, wool, &c.; paper; soap-suds.
   - *b.* Fæcal constituents, especially granules of starch (non-stratified starches generally appear stratified after passing through the bowel); vegetable tissue, which in recent excrement is often stained yellow by bile; residues of muscular fibre, broken up into flakes, of a roundish outline, the transverse stripes occasionally preserved, at least in some pieces; the yellow coloration by bile is often very permanent (according to Gaertner, four weeks). Meat, cooked or raw, which has not passed through the digestive organs is grey,

with the transverse markings much more distinct, as are also the angles of the fragments.—Ova of parasitic intestinal worms. See Figure 83.

3. Animals and plants which live in water or on the sides of wells, pipes, &c.

A minute description of the varied animal and vegetable life in polluted and stagnant wells, &c., is difficult even

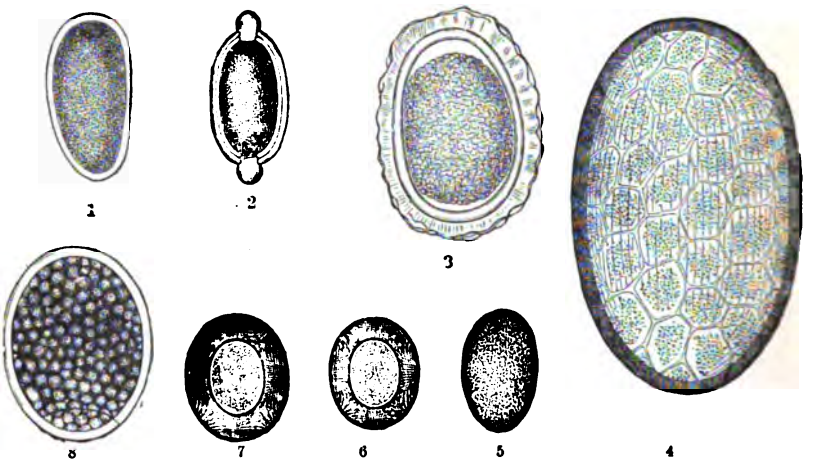

FIG. 83.—Ova of the more common Intestinal Parasites (from Birch-Hirschfeld).

1. Oxyuris vermicularis.
2. Trichocephalus dispar.
3. Ascaris lumbricoides.
4. Distoma hepaticum.
5. Distoma lanceolatum.
6. Taenia solium.
7. Taenia mediocanelata.
8. Botryocephalus latus.

for the specialist, and without great hygienic interest. (See Bibliography.) The following lines may serve for examples:—

A. PLANTS.

1. *Algæ*.—The cells contain a grass green (chlorophyll), blueish green (phycochrome), or brownish yellow pigment.

a. Pigment blueish green (phycochrome); here belongs especially the thread-like genus Oscillaria, which gives off unpleasant odours. *Phycochromaceæ*.

b. Colouring matter yellowish brown. Cells inclosed in a thick armour of silica often beautifully figured. *Diatomaceæ*.

c. Cells containing chlorophyll. Of the numerous families belonging to this group I mention only the following:—

α. Long, tender filaments. Bands or stars of chlorophyll in the cells. *Zygnemaceæ.*

β. Filaments stouter, ramified, without any especial arrangement of the chlorophyll. *Confervaceæ.*

γ. Mono-cellular tissues or complications of few cells, of variable but decided forms, stars, triangles, half-moons, &c. *Desmidiaceæ.*

δ. Mono-cellular, roundish, very simple organisms, sometimes connected by coatings to form simple families. *Palmellaceæ.*

2. *Fungi.*—Cells colourless, brown or reddish, but never green. See § 88.

B. ANIMALS.

1. Small slimy masses with processes "pseudopodia," capable of being extended at will without an envelope (carapace), or with a sheathing composed of the shells of diatoms. *Rhizopoda.*

2. Organisms of a simple structure without distinctly visible organs, but contractile vesicles. Surface covered with a stout cuticle, equipped with cilia, flagella, or ductorial tubes. *Infusoria.*

3. Organisms of a higher structure, consisting of head, trunk, and foot. Discs bearing cilia are arranged round the mouth, and are in almost continuous movement. *Rotatoria (Wheel-Animalcules).*

There are also found certain low worms, some mollusca (especially pond-snails and numerous arthropods, the latter distinguishable by their jointed legs and antennæ). Amongst the arthropods insects are less prevalent than small crustacea.

For a closer study of the higher aquatic flora and fauna, it is useful to allow the water which has been collected to stand in sterilised vessels, closed with wadding.

IV. *BACTERIOLOGICAL EXAMINATION.*

§ 200. The specimens of water are taken, if an immediate examination is possible, in dry sterilised flasks with stoppers of wadding. If they have to be conveyed away, the small bottles with ground glass stoppers, as recommended by Hueppe, are to be preferred; the stopper must be secured in its place with a caoutchouc cap tied round with sterilised paper. Sealing up specimens of water in glass flasks or tubes is not difficult, but scarcely presents any superior advantages. For conveyance, the bottles, first cooled down to 0°, dried with sterilised paper, and wrapped up in the same material, are laid in a wooden box, which is surrounded with finely broken ice, and packed in a sheet-iron box. The

latter is again laid in a wooden case and packed with sawdust.

Specimens of water should be examined at latest at the end of twenty-four hours, for if kept packed in ice the number of microphytes decreases a little, in twenty-four hours from 148 to 126, or from 150 to 115 (Wolffhügel). On the other hand, at the temperature of a dwelling-room the proportion increases rapidly in every water, if the original number was low, *e.g.*, Leone found in the pure Mangfall branch-water at Munich (from a mountain source);

When drawn, 4; after three days, 67,000;
After twenty-four hours, 100; after four days, 315,000;
After two days, 10,500; after five days, 500,000.

If the initial number was very high a decrease may ensue. Rubner has shown (*Arch. f. Hygiene*, xi.) that this increase takes place also in wells, but is not perceptible in consequence of sedimentation.

The enumeration of the germs in 1 *cc.* takes place exactly as directed in § 65. See also the remark in § 58 on the importance of the degree of alkalinity of the gelatine for the number of bacteria. Parallel experiments with nutrient media of different degrees of alkalinity are therefore necessary. Sewage, river water, and the water of suspicious wells should be always diluted with 10 or 100 volumes of sterilised water before plates are made up with $\frac{1}{10}$, $\frac{1}{2}$, and 1 *cc.* For preparing plates for counting the use of agar and of low double capsules is recommended.

In searching for the bacteria of typhus and cholera the investigator should make himself familiar with the procedure described in § 81. Typhus bacteria have of late years been found by numbers of inquirers when water was examined in places where typhus was prevalent (Moers, confirmed by Gaertner; Michael in Johne; Dreyfuss-Brisac and Vidal, Brouardel and Chantemesse, Beumer, Uffelmann, Jäger, and others). In general, all the properties which have been observed in cultures of typhus bacilli have been verified in these cases, so that as far as at present possible their presence must be regarded as proved. In view of the great

difficulty of the accurate diagnosis of the typhus bacillus, Koch, at the International Medical Congress held in Berlin in 1890, has expressed himself very sceptically concerning all these demonstrations, which should always be borne in mind as a warning against the blind over-valuation of the positive results of unqualified hasty observers. See also Cassedebat's sceptical and critical communication (*Annales de l'Institut Pasteur*, 1890, p. 625). Koch has detected the cholera vibrio in a tank at Calcutta surrounded by the dwellings of cholera patients. An investigation for the bacilli of splenic fever would be easy. Various inquirers have found, especially in waste waters, septic microbia, *e.g.*, the bacillus of malignant œdema, of the septicæmia of rabbits and mice, as well as pathogenic staphylococci.

Methods of investigation for the exciting organisms of dysentery and malaria (§ 94) are as yet wanting.

An entire set of travelling apparatus for the bacteriological examination of water has been arranged by Heyroth (*Ann. aus d. Kaiserl. Gesund-Amt*, vii. 381). G. Frank (*Zeit. f. anal. Chemie*, xxx. 305) and Prausnitz (*Centralblatt f. Bacteriologie*, ix. 136) have recommended a very simple portable apparatus. On the conveyance of water for bacteriological purposes, see Pfuhl (*Centralblatt f. Bacteriologie*, viii. 645).

B. Decisions respecting Water.

1. Opinion on a Drinking Water.

§ 201. Let us assume that we have performed upon a sample of water all the more important analyses directed above, and that we have to give an opinion whether the water is good or improper for drinking. Our leading idea must be:—

Water for drinking must contain (1) neither at the time being poisons, nauseating admixtures, nor pathogenic bacteria, nor must it (2) be exposed for the future to the risk of contamination by such substances; lastly (3) it must be of such a quality that it may be drunk willingly, and that its consumption may be pleasant.

Of these three cardinal attributes, Nos. 1 and 2 must always be present. No. 3 we must sometimes dispense with in case of need.

We will now, proceeding from these points of view, submit the results of our several investigations to a critical decision.

1. The temperature must be between 8 and 12° C.; lower temperatures are no great objection, but higher degrees render its use more difficult. Permanent temperatures of from 16° to 20° are almost fatal to the use of a drinking water, as it is then unpleasant.

2. No foreign odour or taste must be present, as, firstly, such waters are repugnant, and are not drunk willingly; and, secondly, as serious pollutions are often thus indicated:—decompositions at the very spot (contaminated well-chambers, decaying wood in the shafts), processes of decomposition in impure surrounding earth, pollution of the subsoil by industrial refuse (tar, saline matter, &c.), leakiness of gas-pipes, &c. The taste of salts of iron is unpleasant to persons not accustomed to it, but not directly injurious.

3. The colour, when seen in deep strata, must be slightly blueish, and the water must be transparent. Suspended sedimentous ingredients must be absent. Deficient clearness gives water a non-appetising aspect, but in many cases such opacity is due to the finest particles capable of passing through any filter, and not injurious to health: particles of clay, of ferric hydroxide, humic substances.

Any turbidity which on macroscopic or microscopic examination indicates a pollution of the water by surface watercourses,[1] slop-water, and more decidedly cesspool contents, stable drainage, &c., render water absolutely unfit for use. Indications of especial value are obtained from the microscopic detection of fæcal matter, muscular fibres, ova of

[1] The present and even the proposed laws on the pollution of rivers in Britain ignore any contamination conveyed into a well through any underground channel. A well in porous soils may thus be safely polluted, so long as the offensive liquids flow into it beneath the surface of the earth.—*Editor.*

intestinal parasites. Such water is, in the first place, to be condemned as disgusting; and, secondly, its consumption is always open to the danger of introducing pathogenic micro-organisms from excrementitious matter. The direct danger of an infection with worms would be small. It is demonstrated that an introduction of the eggs of *Taenia solium* causes the development of cisticerci in man.[1] *Taenia mediocanellata* develops its larvæ from the ova only in the ox, never in man. The ova of *Botryocephalus* are developed alone in fishes, and man becomes infected only by eating the latter. The ova of *Ascaris* and *Trichocephalus* reach their development in man without passing through intermediate hosts. The larvæ of *Ankylostomum duodenale* are developed from ova in foul waters, and infect man on drinking, causing tropical anæmia, the anæmia of miners and brickmakers, &c. *Rhabdonema strongyloides* = *Anguillula intestinalis* (Beray) lives as a larva in man (cause of diarrhœa) in Cochin China, and may doubtless originate from the use of water containing the ova. From the ova of *Distomum hepaticum* there are produced larvæ which develop further in small species of snails (*Limnæus truncatus*). Oxen and sheep are infected by consuming these snails among the herbage. Man is also liable to be infected, though very rarely. In Egypt *Distomum hæmatobium* finds its way into man through the mediation of the water and with similar intermediate hosts. In tropical countries *Filaria medinensis* is introduced into man by means of Cyclops (small crustaceans), and the *Filaria sanguinis hominis* in an unknown manner.[2]

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[1] *Taenia echinococcus* spends its larval state in the tissues of man. This pest seems especially common in Iceland, and is diffused by the agency of dogs. It is abundant where these animals have access to public water-supplies, and where they live in very close companionship with man. The "hydatids," as they are called by medical men, are in reality the scolices of this species. Where water is scarce, as in some parts of Australia, and where men have consequently to drink from stagnant water-holes, hydatidous disease is common, and very dangerous. The spread of *Taenia echinococcus* in Vienna is a different subject, upon which we cannot enter.—*Editor.*

[2] It seems that this scourge is conveyed from subject to subject by the agency of blood-sucking flies, especially mosquitoes. It may also, when very minute, find its way into the bodies of bathers.—*Editor.*

The most recent literature on parasitic worms is to be found in the *Centralblatt f. Bacteriologie.*

4. A moderate proportion in water of Infusoria, Rotatoria, Crustacea, green Algæ, and colourless aquatic fungi renders water loathsome to most people, but to our knowledge is not necessarily injurious. A high and striking proportion of such matter excludes the water from use as very disgusting.

A rich fauna is often a symptom of stagnation, and then it disappears on a more diligent use of the pump. Especial significations have been ascribed to certain groups of organisms and to individual species as regards the quality of water. Here it must suffice us to state that green algæ [1] and diatoms do not prosper in polluted water, whilst certain especially of the smaller infusoria flourish best in such waters along with bacteria. A plentiful development of filamentous aquatic bacteria can indicate both stagnant water and such as is much polluted with organic substances.

It is possible that subsequently pathogenic amœbæ and flagellata, *e.g.*, the exciter of dysentery, may be found in water. Hitherto such a proof is impossible, on account of our defective knowledge.

5. Water is unfit for consumption if any pathogenic schizomycete is detected in it, and it becomes fit for use only if the pathogenic micro-organisms have disappeared again, and if the opportunity for a renewed infection has been obviated as far as possible.

However sceptical we may feel concerning the frequency and the signification of drinking-water as a source of infectious disease, we shall be compelled to accept this view as long as it is not proved that the schizomycetes in question, *e.g.*, the typhus bacilli, act altogether not from the stomach but only from the lungs.

Considering the slender prospect of ascertaining the natural channel of infection in man, in the first place for typhus and cholera, against which all animals hitherto taken for experiment are endowed with immunity, every possible opportunity of infection which we are able to recognise should as far as possible be avoided. It is established that typhus bacilli can generally retain their vitality for a week, occasionally

[1] Green algæ improve the quality of a water. The oxygen which they evolve burns up the organic impurities.

even for several weeks and months, even in non-sterilised water, and consequently in competition with the water-bacteria. Some authors have at times even observed a slight increase, whilst the majority detected merely a more or less rapid decrease of the typhus bacilli capable of development. Experiments with the spirilli of cholera yielded uncertain results, the cause of which lay in part on a varying resistance or adaptation of the organisms employed. Whilst in non-sterilised spring-water the spirilli were often dead at 10° and 20° in twenty-four hours, they remained alive in some experiments even at 10° for ten days (Hueppe), and even twenty days (Gaertner). At the temperature of a living-room Hochstetter observed a survival for 392 days in Berlin tap-water peopled with other organisms. Sewage and other very impure waters agree with them. Bacilli of splenic fever, free from spores, lived only for a few days, but their spores survived in water for months.

The survival of pathogenic bacteria was especially brief in the experiments of Karlinski and Emmerich (*Arch. f. Hygiene*, ix., along with a summary of recent researches on the subject), who infected a well at Munich directly with large quantities of typhus bacilli and the spores of splenic fever. The former had disappeared after three days and the latter after five days, without any previous increase having taken place. The temperature of the water was 10·6°; the well contained moderately impure water, and considerable quantities of broth were introduced along with the microbia.

When Karlinski infected a cistern in various manners with the dejections of typhus patients, no duration of vitality longer than three days was observed. The temperature of the water was 14°, that of the air 26°. Here also no primary increase of the bacteria was perceptible (*Annalen f. Hygiene*, x.).

From enumerations of schizomycetes little can be directly inferred. Water with fewer than 50 bacteria per cubic centimetre may be pronounced poor in microbia; pure waters do not ordinarily contain more than 500 bacteria per cubic centimetre. Bacteria which liquefy gelatine, and those which do not, do not require to be enumerated separately. An abnormally high number of bacteria, such as more than 5000 per cubic centimetre, does not prove the water bad, but indicates the necessity for caution in forming an opinion.

The ground-water is free from microbes, owing to filtration through the soil (unless the impervious stratum is very superficial). Wells, natural or artificial, if properly cased in, contain often only from 0 to 10 bacteria (Munich, Wiesbaden); these few microbes increase, however, very considerably if, *e.g.*, the water is allowed to stand in dwelling-

rooms, and even also in wells which are by no means especially polluted.

But a strikingly high number of microbes will always give rise to the suspicion (especially if many different species are present) that imperfectly filtered water mixes itself from the surface, or through fissures in the well-shaft, which may perhaps be connected with cesspools or other foci of microbes, and that sometimes pathogenic species may penetrate in the same manner into the imperfectly protected well.

At Kaiserslautern (Bokorny, *Arch. f. Hygiene*, viii.), a comparative examination of the water for chemical constituents and bacteria showed that all the well-waters which were chemically good were poor in bacteria; among those chemically bad, some contained many bacteria and some few.

The recent suggestions of Migula (*Centralblatt f. Bacteriologie*, viii. 353) are that the *number* of microbes is to be entirely overlooked. The water may be left, prior to examination, in sterilised vessels for some days. It is examined only for the number of species present, and if among them there are saprophytic bacteria. If ten different species are detected, the water is to be condemned. The proposal to allow the water to stand is very questionable, as some species may increase at the expense of others, and possibly species which were originally single may have increased to masses, whilst pathogenic species may disappear. The number of schizomycetes is also by no means devoid of importance.

6. Waters in which even mere traces of lead are present are unfit for use. Lead takes here the first rank, as we must object even to very small quantities (perhaps even 0·1 to 0·2 *mgrm.* per litre, certainly 0·3 to 0·4 *mgrm.*). Quantities of from 1 to 5 *mgrm.* per litre have to be regarded as large, very large, and exceedingly dangerous on continued use.

Copper, zinc, and tin in small quantities are less formidable. The poisonous character of the metals is discussed in the section on culinary implements.

Potassium sulphocyanide and arsenic have also been found; the former, and possibly the latter, is a proof that the wells have been

polluted with industrial waste waters. Arsenic is found in traces in many waters (*e.g.*, at Leipzig). No instance is hitherto known of a water being found objectionable on account of the natural presence of arsenic. (See Franz Hofmann, *Ueber des Vorkommen von Arsenik in einer städtischen Wasserleitung*, Leipzig, 1878.) Arseniferous mineral waters are of course not here in question. Hydrogen sulphide is certainly a rather powerful poison, but I believe that we do not object to waters which have a slight smell or taste of hydrogen sulphide on account of their poisonous character, but rather because of its offensive flavour. Symptomatically H_2S is of import only when other impurities are simultaneously present which indicate processes of putrefaction.

7. *Organic poisons* have not hitherto been found in waters. If the recognition of ptomaines should be or should become possible, such water would be unfit for use. For the present, waters which require per litre more than from 8 to 10 *mgrm.* permanganate, *i.e.*, from 2 to 2·5 *mgrm.* oxygen for oxidation (containing about 5 *mgrm.* organic carbon and 0·2 *mgrm.* albumenoid ammonia), must be pronounced suspicious for the following reasons:—

1. The "organic substances" (along with the "albumenoid ammonia") form for the present almost the sole basis for the assumption of organic poisons, the occasional presence of which we cannot deny. Attacks of illness after the use of water are certainly more readily explained by the toxic action of ptomaines. On the other hand, water from highly polluted streams, &c., has often been drunk as an experiment without injury, *e.g.*, Emmerich (*Zeit. f. Biologie*, xiv.).
2. The consumption of excessive quantities of permanganate betrays the presence of a strong contamination of the water with soluble organic substances, which it is always possible may penetrate directly from the surface into the well, or may infiltrate laterally.

   Still a number of German cities consume waters which have a high proportion of organic substance; that of Magdeburg contains so much that it consumes 4·0 of oxygen, and that of Posen 7·1.[1]

[1] It would appear that the natives of certain districts have acquired by "natural selection" a kind of immunity against the action of organic substances, certain especial pathogenic microbes excepted. Thus, in some

Humic substances may for the most part be regarded as harmless, but hitherto they are not certainly recognised as such (brown colour). As they are not easily oxidisable, they may be approximately indicated by their high proportion of organic carbon, along with a relatively low susceptibility to oxidation. On the contrary, high oxidability, with a relatively low proportion of carbon, indicates a larger quantity of substances easily oxidised, consequently therefore in part organic bases. If water is allowed to stand, the quantity of permanganate required for oxidation often increases; the activity of the bacteria evidently converting the substances not capable of ready oxidation into such as are more easily oxidised, or nitrites are formed from nitrates, or even inorganic matter is converted into organic substances.

As regards the bacteria of cholera and typhus, it is shown that they require for their increase certain nutrient matters, especially albumenoids. Hitherto, indeed, no certain connection has been traced between the chemical impurity of a water and its capacity to serve for the nutriment of the more exacting microbes; this is not devoid of interest in this respect. Bolton, on mixing the purest possible distilled water with broth, found the vibrios of cholera were not able to increase until 400 *mgrm.* of organic substance were present per litre; the bacilli of typhus were able to increase if 67 *mgrm.* of organic matter were supplied (*Zeit. f. Hygiene*, i.).

8. The chemical constituents of water not enumerated under Nos. 6 and 7 are not poisonous even in the quantities in which they occur in very impure waters. They can therefore never lead to the condemnation of water on toxicological grounds. The following values are exceeded by good potable waters only under exceptional circumstances. The figures (from Tiemann-Gaertner) may therefore serve as a basis for comparison in deciding on the quantities. They do not, however, in any manner represent standard limits in the sense that all waters containing smaller quantities are good, and all with higher quantities are bad.

One litre of good potable water should not contain more than—

Residue on evaporation . . .	500 *mgrm.*
Calcium oxide + magnesium oxide	200 „
(*i.e.*, 20 German degrees of hardness)	
Chlorine	20–30 *mgrm.*

villages in Suffolk the only supply of water is from shallow wells, often separated from cesspools, &c., by merely a few yards of a porous sub-soil. Yet the longevity of the natives is remarkable.—*Editor.*

SO_3	80–100 *mgrm.*
N_2O_5	5–15 ,,
Ammonia . .	at most, minimum traces
Nitrous acids .	
Iron	0·3 *mgrm.*

Harder waters are still quite fit for drinking (the Würzburg water-supply, obtained from a calcareous formation, with 30·4 German degrees of hardness, is by no means unpleasant to the taste), but for domestic and technical uses they are unpractical (see § 203); many people find very soft waters unpleasant to drink, and call it "unrefreshing."[1]

The composition of a water when conducted through leaden pipes is of great hygienic importance. The abundant presence in the water of nitrates and chlorides along with water promotes the solution of the lead. According to Garret (Seventh International Hygienic Congress, London, 1891), the nitrates of the water form with lead loose lead hydroxide, whereby they (the nitrates) are reduced to nitrites. If air is abundantly present, the nitrites pass again into nitrates, and the loose coating of lead hydroxide, which is easily washed away, is formed anew. The presence of copper increases, and that of tin and zinc decreases, the solubility of lead in nitrates. Most important, however, is the proportion of free carbonic acid. Water which decolorises rosolic acid should never be allowed to flow through leaden pipes (Heyer, *Ursache und Beseitigung der Bleiangriffs durch Leitungs-Wasser*, Dessau, 1888). There is first formed a white basic lead carbonate, which is then dissolved in the excess of CO_2 to form lead bicarbonate. The water at Dessau, which dissolves lead, contains, in addition to air, from 34 to 71 *mgrm.* free carbonic acid per litre, but only 2½ degrees (German) of hardness. On the other hand, an abundant proportion of CO_2 protects the lead pipes from attack, because they rapidly become lined with a firmly adhering film of calcium carbonate. Lead containing tin, or coated with tin, is especially

[1] Other persons make the same complaint concerning hard waters.—*Editor.*

attacked by waters rich in carbonic acid. Iron and zinc interfere with the solution of the lead. A high percentage of magnesium salts is not dreaded by any one in Germany. (See also Percy Frankland, Seventh International Hygienic Congress of London, 1891).

A very high percentage of chlorine may generally be interpreted as a distinct indication of a pollution of the water or the soil with the waste products, especially of human economy. Man consumes much common salt; human urine and kitchen-washings are rich in salt (1 litre of human urine = 15 *grm.* common salt; 1 litre kitchen-washings = 1 *grm.* common salt, if we take into consideration only the first, dirtiest water). If salt is given to cattle, their urine may become rich in sodium chloride. A horse excretes daily about 22 *grm.* sodium chloride in 3 litres of urine. If the earth contains deposits of salt, or if it is contaminated with industrial waste waters, this indication loses its value.

Phosphoric acid, if more than a mere trace, must generally be regarded as a certain sign of pollution with urine. The soil retains phosphoric acid so strongly by combination that its appearance is doubly significant. 1 litre of human urine contains 3·5 *grm.* P_2O_5.

It must be especially difficult to draw correct conclusions from the presence of nitric acid, nitrous acid, and ammonia. Formerly the following line of thought was admitted: these substances are formed by the action of organised ferments upon organic substances. Nitric acid represents the ultimate product, and is harmless; ammonia and nitrous acid are intermediate products, which show that the process of nitrification is still incomplete, or that more organic substances are continuously conveyed to the soil than it can transform.

At present we know, especially from the researches of Heraeus (*Zeit. f. Hygiene*, 1886), bacteria which cause nitrates to disappear without leaving a trace; others which reduce nitrates to nitrites and ammonia; others, again, which very quickly oxidise ammonia and nitrites to nitrates. One species even oxidises, in the absence of nitrates, a part of the ammonium salts to nitrites; but if nitrates are present, they are reduced to nitrites and ammonia notwithstanding the most copious access of air.

Another species oxidises ammonia to nitric acid in presence of air, but in the absence of air reduces nitric acid to ammonia.[1]

These processes can evidently take place in the soil as well as in the water, and traces of nitrites and of ammonia have lost their signification. It even seems as if first some ammonia or substituted ammonias must be split off from more complicated nitrogenous compounds (of course by the activity of bacteria) before nitrification can begin. Nitrification generally proceeds so rapidly that the ammonia can rarely be demonstrated.

At the same time it is certain that in fact ammonia and nitrites are scarcely ever found save in strongly polluted waters, perhaps because reducing organisms flourish better in impure water, or because readily oxidisable organic substances retard the oxidation of the ammonia furnished by other bacteria.

A further point of view requires attention. Nitrous acid and ammonia, in the quantities which can ever occur in potable waters, are in themselves not poisonous, but they may be regarded as indications of the possible presence of poisonous basic, nitrogenous products of the splitting up of organic substances which our methods are not yet capable of detecting.

The proportion of iron in the waters of deep wells in the North German plain—0·9 to 3·2 *mgrm.* FeO per litre—renders them unfit for use unless the iron can be reduced to about 0·3 *mgrm.* per litre by some such method as aëration, precipitation of the ferric hydroxide, and removal of the sediment of filtration through gravel. The water then no longer becomes turbid from the deposition of an ochrey matter, and appears even too poor in iron for the growth of the iron-bacteria (Crenothrix) (Proskauer, *Zeit. f. Hygiene*, ix.). When the proportion of iron is higher, unpleasant turbidity from ferric hydroxide and flocks of Crenothrix is

[1] Hence the attempt to calculate the animal matter which may have found its way into water from a determination of the nitric acid, nitrous acid, ammonia, or organic nitrogen present, or from all of them conjointly, is found to be devoid of rational basis, and the entire doctrine of "previous sewage contamination" falls to the ground.—*Editor.*

rarely absent. Such waters have an unpleasant smell and taste, and technically they are almost useless.

When a water—as do most pure spring and tap-waters—fulfils the requirements from 1 to 8, there is no reason for its rejection.

§ 202. For a well-founded recommendatory decision concerning a potable water, something more is required than a single laboratory analysis, chemical and bacteriological. Such an analysis can only show that *at some given time* there was no foundation for the suspicion of unwholesomeness. The following further investigations must give our opinion more certainty, especially with a view to the possibility of future danger.

9. *Inspection of the Well.*—Draw-wells, open at top, are far more exposed to infection and pollution than pump-wells, as also are pit-wells with sides loosely walled, rather than pipe-wells with an iron casing. Surface wells which collect ground-water from a superficial stratum are much more exposed to pollution from above or through lateral fissures, and may therefore be hygienically objectionable, even if they yield for the time being chemically pure water. It is certainly possible that a properly constructed artesian well may penetrate down into a geological stratum which, by reason of its chemical composition, yields water with excessive quantities of solid constituents.

Whilst the water of the Würzburg town-well, which draws its supplies from the anhydrite formation, contains per litre 668 *mgrm.* of solid matter (including 11 *mgrm.* nitric acid), an experimental well in the immediate neighbourhood of Würzburg, driven to the depth of 103 metres, which penetrates into the "Röth" formation (boundary of the bunter sandstone and the shelly limestone), yields a water containing 1724 *mgrm.* of residue and 40 *mgrm.* nitric acid. The water is useless also on account of its bad flavour.

An inspection of any adjacent dunghills and cesspools gives valuable indications as to whether a well will remain pure, or if it is in danger.

10. The well should be examined chemically at different times: turbidities, abundant presence of bacteria, and striking increase of sodium chloride, organic matter, nitrates, nitrites, and ammonia in rainy weather point to the possibility of contamination from without by the afflux of unfiltered water.

11. Uniformity of temperature of the well (mean atmospheric temperature of the locality) throughout the year shows that the water is derived from the deeper strata—in other words, that it has undergone a thorough filtration. Sudden fluctuations of temperature in short intervals of time may indicate a contamination with large quantities of surface-waters.

12. We may also endeavour to trace the causes of an abnormal proportion of micro-organisms by prolonged pumping. If the number of microbia is not thereby decidedly reduced, either (as it has been known to happen) the entire ground-water of the district is rich in microbia, that is, badly filtered, or affluents containing microbes flow into the well, though it is situate in pure ground-water. It is therefore generally a bad indication if the number of microbia is not reduced on pumping. On the contrary, if bacteria which have accidentally penetrated into the well have increased owing to stagnation and a rise of temperature, their number rapidly decreases on pumping in proportion as pure water flows in. It must certainly be conceded that a constancy or even an increase in the number of microbia may appear on pumping, owing to the disturbance of sediments containing microbia.

13. An examination of the pure ground-water of the same geological formation (pure wells and springs of the district) may show that the well in question, notwithstanding its moderate proportion of residue, &c., belongs comparatively to the highly polluted wells of the locality. This does not, however, necessarily decide us to characterise the water as questionable, if all due guarantees are furnished against the introduction of poisons, nauseating substances, and pathogenic organisms, which will generally not be the case. Perhaps in very difficult cases a practically useful decision is not possible without comparative analyses. Especially a decision on the proportion of Cl, N_2O_5, SO_3 presupposes such analyses of pure comparative waters.

For a discussion of the great influence of the geological formation here follow some particulars on pure Franconian

well waters from different strata of the trias. (See Pecher, *Beiträge zur Kenntniss der Wässer aus den geschichteten Gesteinen Unterfrankens*, Würzburg, 1887.)

	1. Bunter Sandstone. Town Well at Lohr.	2. Upper Layer of "Bunter" Sandstone. Trial-boring at Brewery, Würzburg.	3. Wavy Limestone. Brewery, Würzburg.	4. Anhydrite. Town Well at Würzburg.	5. Shelly Limestone. Alands Well near Würzburg.	6. Brown Coal (from the main sandstone).
K_2O	3	16	15	4	4	2
Na_2O	2	100	24	20	6	2
CaO	5	604	251	223	120	80
MgO	...	80	37	58	58	31
Cl	4	64	35	13	14	11
SO_3	...	698	83	186	22	6
CO_2 (combined)	5	84	193	155	142	88
CO_2 (free and half combined)	?	?	220	194	191	222
SiO_2	8	4	7	11	4	4
N_2O_5	...	40	8	11	5	2
Residue on evaporation	27	1724	659	671	376	228

All these waters are potable and fit for use except No. 2. Organic substance and ammonia are not given.

In order to show by example how far the composition of the pure and impure ground waters of entire districts may fluctuate, I quote the following figures, which refer to Bamberg (Mayrhofer, *Arch. f. Hygiene*, iv.).

Composition of pure ground water of the district:—

Residue, 300 to 400.
NaCl, 17 to 30.
Permanganate consumed, 1 to 2.
N_2O_5, 5 to 9.

Contents in the majority of the wells in District II., about—

Residue, 800 to 1400.
NaCl, 130 to 300.
Permanganate consumed, 15 to 50.
N_2O_5, 100 to 350.

If the wells are properly constructed and protected from direct contamination, even these figures are not *per se* sufficient to make the water appear directly injurious to health. But as a matter of course they furnish evidence of a very imperfect removal of refuse, of a very serious pollution of the sub-soil, and when such conditions prevail the structures of the wells, which are often quite decayed and damaged, afford very insufficient protection against direct contamination. To this question the figures quoted above give no definite reply. The microscope and plate-culture have to test the efficiency of the natural filtration. It does not need any further words to show that towns with such conditions will find it their duty to arrange as early as possible for a supply of pure water from without.

It must not be forgotten that there are localities where, on account of the nature of the soil, no pure water can be obtained, *e.g.*, swampy or fenny districts.

Of course a small residue, or altogether trifling chemical impurity, must never induce the expert to recommend a water as good and potable if one of the cardinal properties is wanting. At the utmost, in case of need, we may ignore the requirement that the water should be enjoyable. The only water which is in question at Berlin (river and lake water), has a slight yellowish tint; the water of Zurich (filtered lake water) has very variable temperature. The greater the demand for water in a town, the more moderate, unfortunately, must be our requirements. Still we must always insist that the water, whether furnished from wells or from a central service, must be and remain free from poisons, pathogenic bacteria, and disgusting substances. In the latter case, if the supply is obtained from springs or ground water raised by pumping, the neighbourhood should be carefully protected from pollution; in case of repairs the water of the adits and tunnels concerned is to be drained off. If open waters from rivers and lakes are used, a carefully managed filtration through sand is necessary.

2. Decision on Waters for General Use.

§ 203. Water for such uses must possess all the main attributes of potable water, except that it may not require to be pleasant.

The want of absolute transparence, a slight odour and taste not quite pleasant, and a fluctuating temperature may be tolerated when such toleration is unavoidable. The other requirements must be strictly insisted upon, as such water is often drunk, and must serve for washing (persons, kitchen utensils, clothing, &c.), as also for watering the streets, which is not free from objections if it contains poisonous or pathogenic organisms, or is rich in nutrient matter for the latter. In many large cities it has become customary to distribute into the houses only one kind of filtered water (river or lake water), which may also, for the sake of convenience, be used for drinking, if no very strict demands are made as to taste. At the same time, wherever possible, a number of drinking fountains are arranged in the streets, which supply spring water of a pleasant taste. Cities like Munich, where splendid mountain water can be supplied for general consumption, are few and enviable.[1]

An abundant supply of well-filtered river or lake water is decidedly preferable to a scanty supply of spring water; 70 to 100 litres per head daily is a scanty, 100 to 150 a sufficient, and 150 to 200 an ample supply. But it is now admitted that, with well-fitting mains and proper management, 100 litres per head is sufficient, including the requirements of water-closets (*e.g.*, Berlin).[2]

The demands of technics upon water are naturally different from those of hygiene. They may be here chiefly sketched in order to give

[1] We do not know of any town in Britain where two qualities of water are supplied by the authorities or by companies, though in many instances private persons have provided themselves with artesian wells for their own supply. The municipal water-supplies of Manchester, Glasgow, Leeds, Halifax, Huddersfield, &c., are nowhere excelled in quality, though they ought not to pass through leaden service pipes.—*Editor.*

[2] The average supply of towns in Britain may be stated at thirty gallons daily per head, or approximately 135 litres.—*Editor.*

the warning that technical and hygienic motives must in such questions be kept distinct. According to Tiemann-Gaertner, the following industries require water of the subjoined qualities :—

Steam Purposes.—Hardness, both temporary and permanent, as small as possible to prevent the formation of scale. The bicarbonates may be removed by the cautious addition of lime water until the reaction is faintly alkaline, and the calcium salts may be almost entirely eliminated by adding 1·9 *grm.* soda-ash per cubic metre for each (German) degree of hardness. The efficiency of these additions may be increased by previously heating the feed-water, whilst oxygen and ammonia are expelled. Gypsum produces a much harder and more unpleasant scale than calcium carbonate. Fatty acids and magnesium chloride injure the boiler.

Sugar-Works.—Little nitrates, alkaline carbonates and sulphates.

Paper-Mills, Laundries, Bleach-Works, Dye-Works, Glue-Works.—Soft water, free from iron.

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APPENDIX I.—OPINION ON THE POLLUTION OF RIVERS.

§ 204. In consequence of irrigation, and of earlier and crude processes, as well as of manufactures, tanneries, &c., the water of rivers is in many places seriously polluted, and a decision may be demanded whether a certain degree of contamination justifies sanitary objection.

For such an opinion it must especially be decided:

1. Is the water in the neighbourhood of the city so polluted that it assumes an offensive appearance (becoming turbid, coloured, full of flakes, frothy, &c.), or giving off unpleasant odours, &c.? For a decision it is absolutely necessary to institute an examination of the water, chemical and bacteriological, above and below the city, at various points of the section of the river, at the margins, and in the middle. These examinations should be repeated at different heights of the water, in different seasons, and after different quantities of rainfall.
2. Does the water, though perhaps showing in itself little pollution, deposit putrescible organic suspended substances, which, if disturbed when the level of the water is low, give offence to sight and smell, and suffice to make the contamination appear very bad? This is a very important point in the case of lakes and sluggish rivers (Renk).
3. Is the water rendered unfit for drinking in towns situate lower down along the course of the river in which it has been used as a potable supply? Can possibly poisons, or substances which render water unfit for human consumption (salts from the waste waters of salt-works, colours from dye-houses, &c.), accompanied

by an increase of saprophytic bacteria, and perhaps parasitic fishes, along with disgusting substances, be detected?

4. Does the water lose its value for industrial uses, for street-watering, &c.?

5. Is the character of the water such that fish perish in it?

For a decision on points 1 and 2, observation with the senses is sufficient. Point 3 must be ascertained according to the indications and methods given above. Point 4 must be, in part at least, left to the decision of the technical chemist.

On question 5 we may find a number of statements by Weigelt (*Archiv f. Hygiene*, iii.). See also Kaemmerer (*Bericht der 8ten Versam. Bayer Chemiker in Würzburg*).[1]

It is scarcely possible to give a strict definition to the concept "river-pollution," expressed in chemical and bacteriological figures of limitation, so as to be universally valid. To my knowledge the attempt has never even been made in Germany. The following figures give some indication.

A decree given at Zurich on June 1, 1881 (see Schlatter, *Zeitschrift f. Hygiene*, ix.), orders that no running water must come within 50 *m.* of the outflow of the sewage, and no standing water within 100 *m.*, if it contains per litre more than:

a. So much dissolved or suspended organic matter as requires for reduction 60 *mgrm.* of potassium permanganate, *i.e.*, 15 *mgrm.* oxygen for oxidation.

b. 1 *mgrm.* nitrogen in soluble organic combination.

c. 2 *mgrm.* copper or lead.

d. 0·05 arsenic in any form.

e. 1 *mgrm.* so-called active chlorine (liberated on acidification with sulphuric acid).

f. 1 *mgrm.* sulphur as hydrogen sulphide or as soluble sulphide capable of being decomposed by carbonic acid.

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[1] The susceptibility of fishes to injury from polluted waters varies greatly in different species. All kinds are readily destroyed by lime-water, by sodium sulphate, and especially by bleaching-liquors. Trout can live only in well-aërated waters; but gudgeon, perch, eels, &c., seem little affected by mere animal pollution.—*Editor.*

g. As much alkali as corresponds to the alkalinity of 10 *cc.* normal alkali per litre.

h. So much free acid that the acidity corresponds to 10 *cc.* normal acid per litre.

i. So much tinctorial substance that the water, if placed in a white vessel in a stratum 10 *cm.* in depth, shows a distinct colour.

The decision is especially difficult if it is asked, prior to the installation of an irrigation field, whether the quantity or rapidity of the current and the fall of the river are sufficient to carry down the impurities which are to be washed away, without disadvantage, hygienic or æsthetic. These questions always demand investigation on the spot—investigations in which the engineers have to obtain the most important materials at different times (high and low water, summer heat, breaking up of ice, &c.). The most accurate knowledge attainable concerning the volume of the sewage and its composition is indispensable. The question is particularly difficult when the river is affected by the tides. In general the rule proposed by Von Pettenkofer will never lead us astray,—that ordinary sewage which receives fæcal matters may be admitted without perceptible pollution into a river if (1) the volume of water in the river, at its dry-weather minimum, is fifteen times the volume of the sewage, and (2) the rapidity of the flow of the sewage must not be greater than that of the river. As sewage must have a flow of 0·4 to 0·6 *m.* per second, only rivers with a powerful current are suitable for its reception. Theoretical calculations do not, however, always hold good in practice, and, especially in rivers where the volume of water varies greatly, a certain prediction of the result is possible at most only for those accurately acquainted with these questions.

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Lastly, the reports which numerous towns have caused to be elaborated by commissions of experts on particular cases, *e.g.*, Berlin, Munich, &c.; also those of the English Rivers Pollution Commission.

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APPENDIX II.—EXAMINATION OF AND DECISION ON A WATER-FILTER.

§ 205. A domestic filter of modern construction generally keeps back the *suspended* substances completely. Metallic poisons are for the most part not arrested, and organic substances to a very different degree. The filtering material must never impart an unpleasant after-taste to the water. The micro-organisms of the water are held completely back by few systems for any length of time, and by many very imperfectly. The majority greatly diminish the number of microbia for a time, until these organisms multiply in the

filter itself, and pervade it so that the filtrate is often richer in bacteria than the water which is being filtered.

The manner in which the action of a filter is tested as regards chemical substances, whether dissolved or suspended, follows naturally from what has been said above. For a bacteriological examination the filter is set in action in the prescribed manner, when plates for enumeration are made up simultaneously with the filtrate and the unfiltered water at intervals of a few hours, and then day by day, and the numbers are compared. It must be noticed how the quantity of water passing through the filter alters in the course of time, the yield generally decreasing rapidly, also what is the influence of pressure upon the yield and the capabilities of the filter. The ease with which a filter can be cleaned and restored is also of importance. The sand filters of public water-works seldom yield a filtrate perfectly free from micro-organisms. They act only when the first precipitated matters, and especially a fine bacterial film, have been deposited upon the surface, and the grains of sand have become coated with slimy masses (bacteria and the gelatinised products of their decomposition). It has been assumed that the scanty bacteria present in the filtrate were derived from the deepest strata of the filter, as in 1 *cc.* of the filtrate there are always from 0 to 200 bacteria, alike whether the water being filtered contained 20,000 or 200,000 microbia. In any case the decrease of the microbia should not be calculated in percentages. More than 150 microbia per cubic centimetre in recently filtered water entitle us to pronounce its action unsatisfactory.

For a certain decision upon the efficiency of a filter it is best to work with infusions of microbes which are easily demonstrable, *Bacillus prodigiosus*, and the like; in experiments with domestic filters we may operate with the bacilli of splenic fever and their spores, also with those of typhus, cholera, &c. In this manner C. Fraenkel and Piefke found that in fact sand filters, as they are used, do not completely keep back bacteria (*Zeit. f. Hygiene*, viii. 1).

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APPENDIX III.—EXAMINATION OF AND DECISION UPON ICE.

§ 206. Of late numerous investigations have shown the frequent impurity of ice, both natural and artificial. The chemical examination is effected by wrapping a block of ice in a cloth, breaking it up with the hammer, placing a few fragments in a beaker and melting them on the water-bath. As soon as the ice is melted, the water obtained is examined exactly according to the directions laid down for water. About 2 per cent. of the solid constituents of the original water are said to pass into the ice.

For the bacteriological examination a few fragments of ice are taken as above, passed through a Bunsen flame, and put in a sterilised flask with a plug of wadding. After from fifteen to thirty minutes sufficient water will have been obtained to form plates as in the examination of water. A considerable number of pathogenic bacteria resist even the prolonged action of the low temperatures, *e.g.*, the pyogenic staphylococci and streptococci, the exciters of erysipelas in swine and of typhus. The bacilli of splenic fever and of the septicæmia of rabbits are rather readily destroyed. The ice contains about 10 per cent. of the number of bacteria in the

water from which it was obtained; the number of microbia does not generally decrease on long keeping.

Ice must be judged exactly from the same points of view as water. Even ice for technical purposes (not for direct introduction into beverages, &c.) must be free from poisons, nauseating substances, and pathogenic bacteria.

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APPENDIX IV.—EXAMINATION OF AND DECISION UPON AERATED WATERS.

§ 207. Besides a number of natural waters rich in carbonic acid many artificial waters have come into use. They are prepared by forcing carbonic acid into spring water or distilled water, and have undergone an addition of saline matter, especially sodium chloride.

The examination (according to the methods laid down or indicated in case of water) for alkaline and earthy alkaline salts is as rarely required in the interest of hygiene as is a determination of carbonic acid. If the air has not been previously thoroughly expelled from the water the carbonic acid does not adhere well to the water when poured out, but escapes tumultuously. Carbonic acid obtained from impure materials has often a disagreeable taste of organic substances. Qualitative and quantitative determination of arsenic, lead, copper, tin, and zinc are often required, the methods used being those laid down under "Water" and under "Utensils." These elements may be introduced partly by an imperfect washing of the carbonic acid; in part they may be derived from the plant used in the manufacture, and in part from

improper taps. The taps should, according to law, not contain more than 1 per cent. of lead. Fodor and Steiner detected at Budapest 6·14 *mgrm.* of lead and 6·21 *mgrm.* tin per litre of the contents of the syphons, quantities decidedly very dangerous on prolonged use. Praus detected similar quantities at Warsaw (*Chemiker Zeitung*, 1890, No. 103). Aërated waters with patent stoppers of porcelain, vulcanite, and iron are generally free from lead. Traces of copper are found especially in aërated waters sold by the glass.

The use of aërated water containing pathogenic microbia involves a certain danger to health, though it is a poison for the majority of bacteria. Hochstetter observed that the microbia of splenic fever and cholera were killed by CO_2 in a few hours. The bacilli of typhus, however, may remain alive some days (up to five days),[1] and the spores of splenic fever and of hyphomycetes retain their vitality for months. The resisting power of the harmless saprophytes must vary greatly; whilst Leone constantly observed a rapid decrease of the number of microbia in waters saturated with carbonic acid, Hochstetter often found both in recent and old specimens of artificial seltzer water from 10,000 to 75,000 microbia per cubic centimetre. The energy of the growth of the organisms is retarded by the carbonic acid, so that the plates (sown preferably on agar) must not be counted before the lapse of eight days at the earliest.

A mineral water of a pure taste, free from poison and rich in carbonic acid, cannot be objected to on account of a high number of microbia, unless pathogenic bacteria are present. But legislative enactments are desirable, according to which only waters possessing all the qualities of an unobjectionable drinking-water may be used for the manufacture of aërated beverages. In default of such springs the use of distilled water should be insisted on.

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[1] Helwig traces an epidemic of typhus at Mayence to the use of syphons. *Die Typhus Epidemic von Mainz im Sommer*, 1884. Mayence.

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SECTION IV.

GENERAL PRINCIPLES FOR THE EXAMINATION AND APPRECIATION OF ARTICLES OF FOOD.

A. Examination of the Most Important Constituents of Food.

§ 208. It seems convenient, in order to abbreviate the description of the examination of the several articles of food, to premise the universal methods for the determination of their most important constituents. We recognise as such—

1. The water.
2. The mineral matters (ash).
3. The albumen.
4. The fatty matter.
5. The carbohydrates.

A qualitative examination for the substances is almost universally useless for our purpose and may be omitted. Only quantitative determinations are of value.

The preliminary condition of an accurate analysis is a correct taking of the sample. In general we wish to obtain average specimens, and we must therefore mix the various parts of the substance to be examined as well as possible. Liquids may be mixed by agitation; solids are dried and (with the aid of sieves) comminuted as finely as possible by means of crushing, striking, and grinding, &c., and the portions to be examined are taken from the powder after thorough commixture. All analysis should be effected in duplicate (§ 27).

1. Determination of Moisture, or Desiccation.

§ 209. 1. *Solids.*—We weigh out from 2 to 10 *grms.* of the substance according to the probable amount of moisture and of ash. For a weighing vessel we use by preference two watch-glasses ground to fit each other, and retained in their places by an elastic clamp, or—indeed always if a determination of ash is to be included—a light, flat capsule of platinum or thin Berlin porcelain. The substance is spread out as much as possible. The recipients (the watch-glass apparatus opened) are placed for four or five hours in the drying-closet at from 100° to 105° (§§ 8 and 61), weighed at the end of this time after having been allowed to cool in the exsiccator. The weighing is repeated about every hour until the loss of weight from hour to hour does not exceed from 1 to 2 *mgrm.*

2. Liquids are either directly evaporated down according to the instructions given in § 1 (this is always done in case of albumenous liquids), or they are mixed with sand (previously ignited), and evaporated on the water-bath, stirring with a glass rod, as it is described in detail in the directions for milk. In this manner we avoid the formation of lumps, which may inclose water.

If water has to be determined in presence of alcohol, we ascertain both water and alcohol in one sample, and in another the alcohol alone. The water is then found as difference.

A similar but more difficult case is when ethereal oils, volatile fatty acids, &c., are present along with water; the end may often be obtained by drying the substance over sulphuric acid in the exsiccator without the application of heat. But even if we can exhaust the air this process is tedious, often lasting for days until all the water is removed. No general instructions can here be given, as a special procedure may be required in each case.

2. Determination of Ash.

§ 210. For the determination and examination of the non-combustible inorganic (mineral) constituents of animal or vegetal substances, whether they are solid or liquid, we generally use the dry residue left after the determination of moisture. This represents the sum of all the fixed con-

stituents, organic and inorganic, whilst the ash represents the latter only.

As it has been indicated under the determination of moisture, we take of solids from 2 to 10 *grms.*, according to their larger or smaller proportion of ash ; of milk about 10 *cc.* ; of wine and beer 50 *cc.*, which, however, must be weighed. As the values to be determined are frequently not referred to the original substance with its fluctuating proportion of moisture (*e.g.*, flour, spices, &c.), but to the dry residue, the determination of moisture must be effected previously.

The flat platinum capsule[1] with the weighed dry residue is placed upon a clay triangle (§ 4), and a small gas flame is first cautiously moved to and fro underneath it in order to prevent spirting or irregular frothing up and running over (in case of wine or beer). It is advisable to let the charring extend gradually inwards from the margin. If the gases given off take fire, this is not injurious; they are allowed to burn quietly on, the operator being thus less annoyed by offensive fumes. Many substances burn at once to whiteness; others, which are rich in phosphates or potassium salts, include unburnt particles of carbon so firmly that direct incineration is impracticable.

Too high or too prolonged a temperature might at most volatilise the alkalies, thus causing the values obtained to be too low ; in this case (*e.g.*, beer, flour, bread) we ignite only until the carbon glows no longer perceptibly. It is then allowed to cool uncovered (the carbon thus attracts oxygen) ; the mass is crushed with a stout platinum wire, or a glass rod melted round at the end. During this operation the capsule should be set upon a sheet of glazed paper, so that any particles thrown out may be swept back into the capsule with the feather of a quill. It is then ignited anew over the flame, the incineration being expedited by stirring with the stout platinum wire. Even so a perfectly white ash is in many cases not yet obtained. It is then necessary after cooling to dissolve the salts which inclose the particles of carbon in a little water. If but little matter remains undissolved the capsule is placed in an inclined position upon a boiling water-bath until the chief part of the water is evaporated, and the carbon has attached itself rather firmly to the capsule. The solution of the ash is then allowed to flow away from the carbon by turning the capsule cautiously, with occasional

[1] In default of platinum capsules, porcelain crucibles are used for determining the ash, as also when the substance contains ingredients which might injure platinum crucibles (§ 4).

pauses, and it is then evaporated to dryness at the opposite side of the capsule, when the carbon is again cautiously ignited on an open flame.

If the quantity of the substance insoluble in water was considerable, it is passed into an Erlenmeyer flask along a glass rod, through a filter the proportion of ash in which is known; the rod, funnel, &c., are rinsed with boiling water, and the residue, together with the filter, is returned to the capsule. If on once more heating no white ash is obtained, washing and gentle ignition are repeated until that end is effected. The capsule is then allowed to cool, placed upon a water-bath (a porcelain ring or a filter-paper being placed beneath), and the filtrate and washings are gradually added to the solution of the ash; the apparatus used is rinsed with distilled water; the total liquid is evaporated to dryness, as far as practicable, upon the water-bath, and the residue is then ignited over a small flame, which is moved to and fro and gradually increased. If any spirting occurs the entire determination must be repeated.

If—which often happens—the ash is very hygroscopic, it must be weighed as rapidly as possible after a brief ignition; the weight found on the first weighing must be at once laid on the scale-pan.

3. Determination of Albumenous Substances.

§ 211. Hitherto methods for the direct determination of that important constituent of foods, albumen, in complicated mixtures, have been wanting.[1] If we set aside a few cases, *e.g.*, milk, in which the albumen may be directly precipitated and weighed (see Milk), we are, as a rule, compelled to determine the nitrogen instead of the albumen. Animal albumen contains on an average 16 per cent. nitrogen. If we therefore multiply the nitrogen found by 6¼ the product is albumen. If we are seeking vegetable albumen, which contains 16·66 nitrogen, we multiply with six only. This is always done in physiological researches, but food-chemists generally use the factor 6·25 indiscriminately. Unfortunately we commit grave errors if we at once calculate the total nitrogen of foods as due to albumen. This is permissible in case of meat, milk, and cereals, which contain, along with abundance of albumen, only small quantities of other nitrogenous constituents

[1] Indications for these circumstantial investigations may be found in—
RUBNER. *Ueber fluid meat. Zeit. f. Biologie.* 1879 and 1880.
STUTZER. *Jahrbuch für Landwirthschaft*, 1881, p. 473.
UFFELMANN. *Ueber den Eiweissgehalt und die Verdaulichkeit der essbaren Pilze. Arch. f. Hygiene*, vi.

(extractive matters). It is incorrect in case of potatoes (in which 40 per cent. of the nitrogen must be referred to amidic substances, solanine, &c.), in fungi (in which from 20 to 30 per cent. of the nitrogen is present as amidic substance), in cheese, &c.

In extract of meat, in extracts of beer, wine, soils, sewage, &c., it is best not to recalculate the nitrogen, but to state it simply as such.

§ 212. For the determination of the nitrogen[1] two methods are customary:

1. *Method of Will-Varrentrapp.*—The process depends upon the principle that the nitrogen of all nitrogenous substances is converted into ammonia by ignition with soda-lime (caustic soda + caustic lime). The ammonia volatilised is absorbed in sulphuric acid, and determined by titration. As this method requires an expensive combustion-furnace, takes a considerable time, requires blank check experiments, consumes many combustion-tubes, and as the accurate titration of the liquid is often rendered difficult by the presence of coloured products, and as it possesses no advantages over the second process about to be described, these indications must suffice.

2. *Kjeldahl's Method.*—This method, which has been known only for a few years, has quickly become naturalised in all laboratories, and is now almost exclusively practised. Innumerable slight modifications are in use. We have here taken as a foundation *Proskauer and Zülzer* (*Zeitschrift f. Hygiene*, vii.).

Principle.—All the nitrogen in the dry, finely pulverised

[1] The nitrates are not converted into ammonia by either method. If we wish to determine the nitric nitrogen by Kjeldahl's method, we reduce the nitric acid with zinc and dilute sulphuric acid to ammonia (see § 197*a*), or we determine in one sample the nitrogen, exclusive of the nitrates, and in another the nitrates. As, if much nitrates are present in Kjeldahl's process, a small part is converted into ammonia, we boil in this case the substance first for one or two hours with dilute sulphuric acid in a porcelain capsule, so as to expel the nitric acid. Nitrates occur relatively seldom in hygienic investigations, except in soils, water, and meats salted with nitrates.

substance, or in the dry residue of liquids, passes gradually but completely into ammonia on heating with concentrated sulphuric acid. It is expelled by supersaturation with soda-lye, and received in $\frac{1}{5}$ normal sulphuric acid.

Reagents.—1. Concentrated sulphuric acid, absolutely free from nitrogen. It is best to use the following mixture: 800 *cc.* pure concentrated sulphuric acid, 200 *cc.* fuming sulphuric acid, in which there are dissolved 100 *grm.* phosphoric anhydride. A few blank experiments, made according to the method described below, prove the absence of nitrogen, or determine the quantity existing in 20 *cc.*, which must then be deducted from the results obtained each time.

2. Fused anhydrous copper sulphate.
3. Mercury.
4. Potassium sulphide solution, 40 *grm.* per litre. To be prepared at least three hours before use, so that any undissolved portion may have time to subside.
5. Soda-lye, 500 *grm.* per litre.
6. $\frac{1}{5}$ normal sulphuric acid.

There are further required long-necked decomposition flasks, holding about 150 *cc.*, made of well-annealed potash-glass, for boiling the acid mixture, and roomy (750 *cc.*) Erlenmeyer distillation flasks. All the flasks are preferably provided with a *matt* escutcheon for affixing remarks.

Execution of the Process.—The weighed mass, finely powdered—$\frac{1}{2}$ *grm.* if rich in nitrogen (meat, or extract of meat), 1 *grm.* if of mean strength (flour, bread), and 2 to 5 *grm.* if poor in nitrogen (starch)—is put in a boiling flask, 20 *cc.* of the acid mixture are added, and also about 0·5 *grm.* anhydrous copper sulphate, and about 1 *grm.* metallic mercury. These quantities are only weighed once; in subsequent operations they are estimated with the eye. The flask is set upon a wire grating, with its neck in a slanting position, and heated with agitation for thirty to forty-five minutes with a small flame (preferably with the rose-burner) until the substance is dissolved, and then for about three hours longer, or until the solution becomes clear, colourless, or of a pale

yellowish or greenish colour. If too strong a heat is applied before the substance is dissolved, a loss of nitrogen may very readily take place.

If nitrogen is to be determined in liquids, weighed quantities are introduced into the empty decomposition flask, and they are evaporated as nearly as possible to dryness with the addition of a few cubic centimetres of dilute sulphuric acid. Of milk and beer we take about 20 *grm.*, of wine 50 to 100 *grm.*, of water at least 200 *grm.*, proceeding afterwards as it has been directed for solids. For N_2O_5 see § 212.

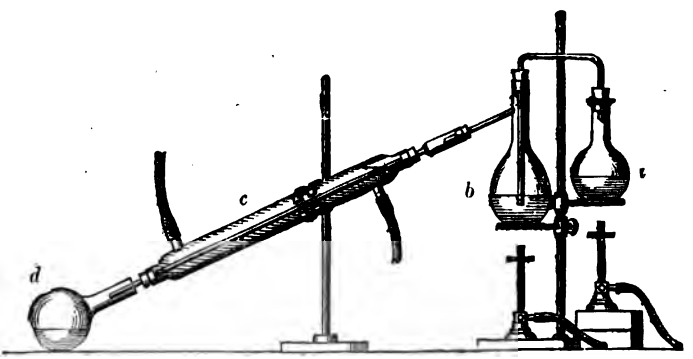


FIG. 84.—Determination of Nitrogen with the Use of Landmann's Apparatus (the flask *b* must be fitted with a bulb, as in Fig. 85).

When the colour has disappeared the liquid is allowed to cool slightly, and the contents of the decomposition flask are then poured into the capacious Erlenmeyer distilling flask, and the former is twice rinsed out with water. The liquid, which has been again heated by the addition of water (about 150 *cc.*), is allowed to cool slightly, and soda-lye is added in slight excess. It must have been previously ascertained how much lye is required to turn the acid which has been used to the alkaline side; this quantity (on working according to the above instructions, about 76 *cc.*) is added in two portions, at first 60 *cc.*; the mixture is allowed to cool again, and there are then rapidly added 16 *cc.* of lye and 40 *cc.* solution of potassium sulphide, by which the mercury is precipitated. A few fragments of zinc are then quickly

dropped in, and the apparatus is closed. The pieces of zinc serve to moderate the bumping on subsequently heating.

§ 213. The ammonia liberated by the soda-lye is then distilled off in various manners, but the receiver is always charged with 50 *cc.* of $\frac{1}{5}$ normal sulphuric acid.

The majority of chemists distil now without a condenser. Immediately after closing the apparatus, the flask is heated upon the wire-grating. It is important (Fig. 85) for the

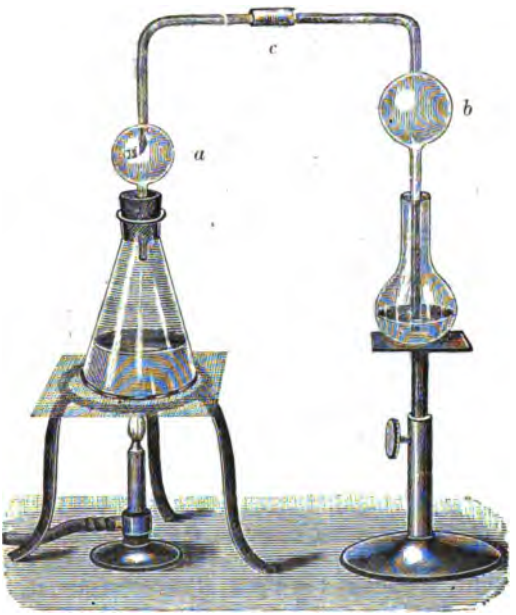


FIG. 85.—Kjeldahl's Determination of Nitrogen without a Condenser.

distillation to have a bulb appendage (*a*), which prevents the soda-lye from spirting, which it sometimes threatens; secondly, the descending limb must be very wide, and it must also be fitted with a bulb, so as to prevent the sulphuric acid in the receiver from being sucked back into the distillation flask. This accident is, of course, threatened as the heated distillation flask begins to cool. At the commencement of the distillation the liquid generally bumps violently a few

times, and then boils quietly. Towards the end of the process, when the liquid is evaporated down to about one-half, cracking bumps occur afresh, and give warning that the process is completed. When the experiment is at an end, the piece of flexible tube *c* is loosened, and the bulb-tube *b* is rinsed into the receiver with a little distilled water.

Proskauer and Zülzer recommend the ammonia to be driven off by means of an apparatus constructed as in Fig. 84. *b* contains the alkalised sulphuric acid; *a*, distilled water. *b* is slightly heated over a small flame; *a* is caused previously to boil briskly. The distillation-tube must plunge into the recipient acid (Fig. 84 shows this incorrectly). The distillation is continued until about 200 *cc.* have collected in the recipient flask.

After the experiment is completed we titrate back in each case with $\frac{1}{5}$ normal soda-lye, using as indicator tincture of litmus or rosolic acid.

The calculation is best shown by an example :—

There was taken for examination 1 *grm.* of rye-flour.

In the receiver there were 50 *cc.* of $\frac{1}{5}$ normal sulphuric acid, which on titration with rosolic acid consumed only 42·3 *cc.* of $\frac{1}{5}$ normal soda-lye instead of 50 *cc.* A quantity of ammonia has therefore been absorbed equivalent to $50 - 42{\cdot}3 = 7{\cdot}7$ *cc.* $\frac{1}{5}$ normal soda-lye.

1 *cc.* normal soda-lye (see § 27) contains $\frac{40}{5}$ *mgrm.* NaOH, and corresponds to $\frac{17}{5}$ *mgrm.* ammonia or $\frac{14}{5} = 2{\cdot}8$ *mgrm.* nitrogen. The decrease in strength of 7·7 *cc.* signifies, therefore, the proportion of $7{\cdot}7 \times 2{\cdot}8 = 21{\cdot}56$ *mgrm.* nitrogen, or a proportion of albumen of $6{\cdot}25 \times 21{\cdot}56$ *mgrm.* As this quantity is contained in 1 *grm.* of the substance, the proportion of albumen is 13·475 per cent.

4. Determination of Fat.

§ 214. *Principle.*—The fatty matter can in most cases be extracted from the dried substance by means of ether, and may then be either directly weighed after the expulsion of the ether, or the loss of weight of the substance extracted with ether is determined after previous desiccation.

The process is conducted with the apparatus of Szombathi-Soxhlet.

The ingenious construction of this apparatus is shown in the accompanying figure (Fig. 86). It consists of three parts: a wide-necked flask, *a*, which is weighed when empty, and is filled to a half or two-thirds of its capacity with ether; further, the extraction apparatus strictly speaking, *d*, and the refrigerator, *k*, in which the vapours and the ether are condensed as they ascend out of the flask, which stands upon a narrow ring over a water-bath boiling gently.

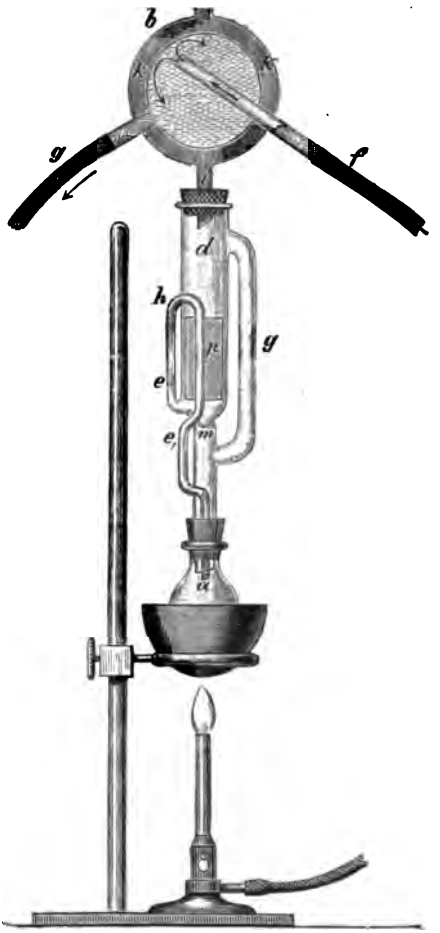


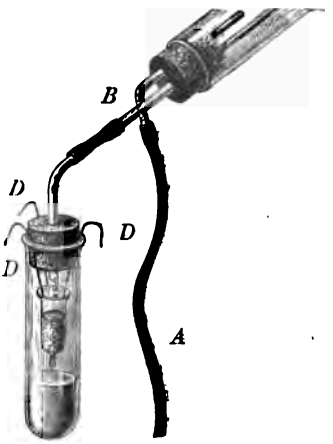
FIG. 86.—Apparatus of Szombathi-Soxhlet, with a Metal-cooler, according to Soxhlet's design. (The water-bath is represented too small.)

As a refrigerator there is often used Soxhlet's double ball of nickelised sheet-iron as here figured, which is traversed by a current of water as shown in the figure. In its stead we may place a Liebig condenser in a steeply ascending position upon the flask. In any case a metallic refrigerator which has never been employed, or which has been out of use for some time must be carefully cleansed with water, alcohol, and ether, and then dried before it is used in an experiment. The latter is packed by rolling twice round a cylindrical cork (rather thinner than *d* in Fig.

86) a piece of filter-paper freed from fatty matter, allowing a piece of the coil formed, corresponding to its diameter, to project, and bending this round to a bottom on closing the packet. The cork is then taken out, the substance introduced into its place, and the capsule is closed by folding down the prominent margin. There is thus formed a package closed on all sides (*p*), and introduced into *d*. The track of the ether vapours is as follows: they do not pass directly from *a* at *m* into the extraction vessel (which is here closed), but through a wide tube, *e*, directly into the upper part of the extraction space in which *p* is placed. From here they pass into the cooling-tube *i*, where they condense into drops, and fall in rapid succession down upon *p*, gradually washing round the package from all sides and finally covering it. At the same time the ether in the extraction space rises in the narrow tube *e*, until arriving at its curvature at *h* it flows down to e_1, and all the ether present in the apparatus suddenly discharges itself by syphon action, together with the fat which it has dissolved. This play repeats itself about every five minutes; the package is constantly wasted by fresh ether, and the fat collects more and more completely in the wide-necked flask. Of course caoutchouc stoppers must not be used, but corks which have been previously freed from the fatty and resinous matters which they contain by prolonged steeping in ether, or preferably by treatment in a large extraction apparatus. The filter-paper used must also be freed from fat in a similar manner.

Substances rich in fat must often be extracted for six hours and longer; for those poor in fat three hours may suffice. In order to ascertain whether the extraction is complete we substitute a second ether flask for the first one; when it has been in action for ten minutes no increase of weight beyond 1 or 2 *mgrm.* must be found after the evaporation of the ether. This check must not be neglected. It is very easy to work with the apparatus, but care must be taken not to heat the water-bath too strongly. As sometimes the paper case does not prevent the passage of all the finest particles of the substance, whereby the lower narrow tube becomes stopped, it is advisable to place at the bottom of *d*, below *p*, a plug of asbestos, or of wadding freed from fat, as a filter.

In order to determine the fat when the extraction is complete, the ether may be firstly evaporated away from the solution of the fat and the flask weighed. For this purpose the flask is held cautiously in a vessel of hot—not boiling—water, remote from any flame, At first it is well to shake the flask continually round and round. If the flask is not filled to a great extent with the ethereal solution of fat, it is often most convenient to expel the ether by simply placing the flask upon a support of paper at the top of the evaporation closet. When the drying is completed the flask is set in the drying-closet at the heat of 100°; it is weighed for the first time after two hours, and then every half hour until the weight becomes constant. If the drying-closet becomes hotter there occurs a more profound decomposition of the fat; a slight error in this direction is occasioned even by drying at 100°. Secondly, the fat may be indirectly determined from the loss of weight which the package undergoes if carefully dried before and after the experiment. It is advisable to use both methods; the results of careful work agree well if the paper case closes properly.



Extraction Apparatus of Medicus'

Many simpler apparatus have been proposed; that of Medicus (Fig. 87) is good, not very fragile, and inexpensive. The substance is placed in the interior tube, which opens downwards, and is constantly traversed by the ether which condenses and drops upon it. The outer tube, charged with a little ether, rests with its top upon a small, narrow ring of the water-bath. *D*, *D*, *D* are three wire hooks. The tube *B* serves to condense the ether; it traverses a Liebig's refrigerator, the water of which is conveyed away by *A*. When the experiment is complete the ether is poured from the outer tube (which is taken off) into a weighing-glass, and the fat is weighed after the evaporation of the ether.

5. Determination of the Carbohydrates.

1. IDENTIFICATION OF THE VARIOUS CARBO-HYDRATES.

§ 215. It is rarely possible to determine the carbohydrates as difference by subtracting from the dry substance the weight of the ash, the albumen, and the fat. Approximate values may be obtained for commercial starch, fine flour, and fine bread. A direct determination is mostly necessary, which may prove especially difficult if several carbohydrates have to be simultaneously determined.

The following conspectus gives us a general view of the most important carbo-hydrates :—

1. Soluble in water :
   - *a.* Soluble in alcohol :
     - *α.* Reduce Fehling's solution at once :
       Dextrose = grape-sugar ($C_6H_{12}O_6$).
       Levulose = fruit-sugar ($C_6H_{12}O_6$).
       Maltose = malt-sugar ($C_{12}H_{22}O_{11}$).
       Lactose = milk-sugar ($C_{12}H_{22}O_{11}$).
     - *β.* Reduce Fehling's solution only after inversion :
       Saccharose = cane-sugar ($C_{12}H_{22}O_{11}$).
   - *b.* Precipitable by alcohol :
     Dextrine and gum ($C_6H_{10}O_5$) (reduce Fehling's solution only after inversion).
     Some dextrines give a red colour with a dilute solution of iodised potassium iodide.
2. Insoluble in water :
   - *a.* Convertible by diastase into maltose, and by boiling with acid into dextrose. Swells up to paste with hot water ; coloured blue by solution of iodine in the cold ; the colour disappears on heating, and returns at the temperature of a dwelling-room.
     Amylum = starch ($C_6H_{10}O_5$).
   - *b.* Not convertible into any kind of sugar by diastase, but by very prolonged boiling with dilute acids.
     No direct coloration by iodine, though coloured after the action of concentrated sulphuric acid (by the formation of starchy substances).
     Cellulose ($C_6H_{10}O_5$).

By inversion we understand the conversion of non-reductive carbohydrates by boiling with dilute acids (or by ferments) into sugars of the formula $C_6H_{12}O_6$, which directly

reduce Fehling's solution to a yellow cuprous hydroxide, $Cu_2(OH_2)$, or to a yellowish red cuprous oxide, Cu_2O.

Fehling's liquid,[1] according to the description of its inventor, was a solution of copper hydroxide obtained by means of tartrates, but has the disadvantage that it is partially decomposed if preserved for a short time. The three components of Fehling's liquid are now always preserved separately.

a. Aqueous solution of 34·64 *grm.* purest copper sulphate ($CuSO_4 + 5H_2O$) made up to 500 *cc.*

b. Aqueous solution of 173 *grm.* Seignette's salt (sodium potassium tartrate) made up to 400 *cc.*

c. Aqueous solution of 250 *grm.* purest caustic soda made up to 500 *cc.*

Small quantities are mixed immediately before use in the proportion of 80 *cc.* solution of sodium potassium tartrate + 20 *cc.* soda-lye + 100 *cc.* of copper sulphate solution, always in the order given.

2. QUALITATIVE RECOGNITION OF THE SEVERAL CARBOHYDRATES.

§ 216. The substance (pulverised if necessary) is extracted with water, and the filtrate is examined, as to its taste and its behaviour, with Fehling's solution, which is diluted with an equal volume of water, boiled in a test-glass, and kept for about six minutes at about 100°, along with a few cubic centimetres of the filtrate. If cuprous oxide separates out the kind of reductive sugar is ascertained by means of the polarising apparatus in a manner not to be here described. If the liquid remains clear and blue no reductive species of sugar are present. The residue of the filtrate, which may contain cane-sugar, dextrine, and gum, is evaporated almost to dryness upon the water-bath, adding gradually absolute alcohol whilst stirring in order to separate dextrine and

[1] The most recent experiments on determining the sugars by boiling with Soldaini's liquid (copper potassium carbonate) need merely be mentioned, as a final decision has not been reached, whether the process has essential advantages which outweigh slight defects.

gum, whilst cane-sugar remains in solution, and is recognised by its sweet taste after the evaporation of the filtered solution; or by redissolving in water and boiling for a time with a few drops of hydrochloric acid, neutralising the acid with soda-lye, and then, as before, adding a few cubic centimetres of the liquid to boiling Fehling's solution.

In a similar manner the dextrine or gum, eliminated by means of alcohol, may be dissolved in water and converted into reductive sugar by prolonged boiling, or by heating under pressure. Starch and cellulose, which are not soluble in water, are also inverted by boiling in water (the latter very slowly). Starch alone is saccharified by diastase (in an extract of malt) at temperatures below 70°, whilst cellulose remains undissolved.

Thus the several carbohydrates may be recognised if simultaneously present, though certain bodies must be absent (*e.g.*, sulphurous acid, certain constituents of urine), since they also reduce Fehling's solution.

3. QUANTITATIVE DETERMINATION OF THE CARBOHYDRATES.

§ 217. **General Preliminary Remarks. Principle of the Methods.**—For a quantitative determination all the carbohydrates (with the exception of cellulose), if not directly reductive, are converted into reductive sugars in the manner above mentioned, and the latter are determined volumetrically or gravimetrically with Fehling's solution, or they are determined optically.

The principle of the volumetric method is: we ascertain how much solution of sugar is exactly sufficient to reduce 50 *cc.* of Fehling's solution. In the gravimetric method we weigh the cuprous oxide, separated from an excess of boiling Fehling's solution by a known quantity of solution of copper, after its conversion into metallic copper.

When working with Fehling's solution, whether volumetrically or gravimetrically, the following points must always be kept in view:—

1. Of each kind of sugar 1 *grm.* reduces a perfectly deter-

minate weight of copper, which is characteristic for that kind of sugar.

2. This quantity depends not alone on the kind of sugar, but on the accurate conditions of the experiment (concentration of the solution of sugar, quantity of Fehling's solution, duration of boiling, &c.). The defined experimental conditions are therefore to be most carefully observed.

If we work according to Soxhlet, always with solutions of sugar containing as nearly as possible 1 per cent. of sugar, and with undiluted Fehling's solution, the following numbers hold good:—

½ *grm.* Sugar.	Reduces *cc.* of Fehling's Liquid.	50 *cc.* of Fehling's Liquid.	Represent Grammes of Sugar.
Grape-sugar . . .	105·2	Grape-sugar . . .	0·2375
Invert-sugar . . .	101·2	Invert-sugar . . .	0·2470
Levulose	97·2	Levulose	0·2572
Lactose	72·0	Lactose	0·3380
Maltose	64·2	Maltose	0·3890

The highly dilute solutions with which we have to work in chemical determinations of sugar render careful manipulation especially a duty; all errors are largely multiplied.

The different kinds of sugar reduce boiling Fehling's liquid with different speeds; thus it is necessary to boil—

For grape-sugar	2	minutes.
„ inverted sugar	2	„
„ levulose	2	„
„ maltose	3 to 4	„
„ lactose	6 to 7	„

All closer details on the methods are to be found under the several sugars; in the fullest detail under grape-sugar, which serves as a general example.

§ 218. **Grape-Sugar (Dextrose).**—*Determination of Grape-Sugar by Titration.* (For the principle involved see § 217.)

By a preliminary trial which we make previously with an aliquot part of the known volume of available liquid, we find that, *e.g.*, 16 *cc.* of sugar solution are required to decolorise

50 *cc.* of Fehling's solution. Now a solution of grape-sugar contains 1 per cent. when 23·7 *cc.* have to be used for 50 *cc.* of Fehling's solution; we must therefore dilute every 16 *cc.* of the original solution to 23·7 *cc.* The preliminary trial is carried out in the same manner as the actual determination.

Performance of the Analysis.—To 50 *cc.* of Fehling's solution, which are boiled in an undamaged porcelain capsule upon a wire grating, we add the quantity of dilute sugar solution which is expected according to the preliminary trial (*e.g.*, 24 *cc.*), boil for two minutes, and then pour quickly the hot contents of the capsule upon a folded filter. If the filtrate is blue or blueish green, too little solution of sugar has been added. If it is yellowish, a portion of copper may still exist in solution. In order to detect dissolved copper, a part of the yellow filtrate is acidified with acetic acid and two drops of a solution of potassium ferro-cyanide. A faint reddish brown coloration indicates a small proportion of copper, a strong reddish brown coloration proves the presence of a considerable amount of copper, *i.e.*, too small an addition of the solution of sugar. If no change of colour is produced by the potassium ferro-cyanide, the quantity of solution of sugar added has been either accurate or in excess. New experiments have therefore to be made until the right quantity is reached. The procedure is best shown by an example.

Example (according to Soxhlet):—
23·0 *cc.*: filtrate a blueish green, *i.e.*, far too little sugar added.
24·0 *cc.*: filtrate greenish, *i.e.*, rather too little sugar added.
25·0 *cc.*: filtrate yellow, no reaction for copper, *i.e.*, either the right quantity of sugar or too much sugar has been added.
The true value is therefore intermediate between 24 and 25.

Analyses with intermediate quantities gave for—
24·5 *cc.*: filtrate yellow, strong copper reaction.
24·7 *cc.*: filtrate yellow, faint copper reaction.
24·8 *cc.*: filtrate yellow, no copper reaction.
Consequently, 24·75 is the correct value; *i.e.*, there are 0·2375 *grm.* grape-sugar in 24·75 *cc.* of the solution.

The calculation is very simple. 24·75 *cc.* of solution of grape-sugar contain in our example 0·2375 *grm.* of grape-

sugar, since they reduced 50 *cc.* of Fehling's solution. If the quantity of the sugar solution before dilution was 200 *cc.*, it was in our example $\frac{200 \times 23{\cdot}7}{16}$ *cc.* after dilution; the quantity of grape-sugar was $\frac{200 \times 2{\cdot}37 \times 0{\cdot}2375}{16 \times 24{\cdot}75}$ *grm.* = 2·84 *grm.*, or 1·42 per cent.

The results of the volumetric process agree with those of the gravimetric analysis only if carried out with consummate accuracy, as attained only by prolonged practice. In addition, the many preliminary trials render the process somewhat tedious, so that its only advantage, as compared with the gravimetric process, lies in the fact that the balance is dispensed with. Other more convenient methods of titration yield only approximate values (see Wine).

§ 219. **Gravimetric Determination of Grape-Sugar** (according to Allihn).—For the principle of the method see § 217.

Performance of the Analysis.—The solution of sugar is diluted in the manner described in § 218, so that it does not contain more than 1 per cent. We put 60 *cc.* of Fehling's solution and the same volume of water into a porcelain capsule holding 300 *cc.*, heat it to ebullition, and add 25 *cc.* of the solution of sugar. We now boil for five minutes, and filter, whilst hot, through an asbestos filter. The asbestos filter-tubes (which may be purchased) are to be prepared from pieces of potash glass tubing (combustion tubing) 10 *cm.* in length, drawn out below to one-third of their width. The lower quarter of the wider portion of the tube is plugged rather firmly with recently ignited asbestos, having a long fibre: some glass wool may be put below the asbestos. The plug must not be too compact, lest the filtration proceeds too slowly, and not too loosely, lest cuprous oxide passes through.

Soxhlet's Filter-Tube.—The space *C* in the figure is too short; it must be, in proportion, twice as long as in the figure. A funnel, *A*, is placed at the top, in order to pour

in the solution of copper. The drawn-out end is fixed, by means of a caoutchouc stopper with two perforations, into a flask, from which the air is extracted by means of a water air-pump (Fig. 37). Thus the filtration is effected rapidly at a reduced pressure. The solution over the copper is first poured in, as nearly alone as possible; the cuprous oxide is washed with cold water, which is poured through the filter. Lastly, the cuprous oxide is brought upon the filter by means of a glass rod tipped with caoutchouc. Until the end of the operation, or until the flexible tube of the air-pump is taken off, liquid must always stand above the level of the asbestos plug, which prevents the cuprous oxide from breaking through. When the last trace of copper sulphate has been washed away with water, alcohol is poured upon the filter twice, and then a little ether; the contents of the filter are then desiccated in the drying-closet.

FIG. 88.—Soxhlet's Filter-Tube.

The cuprous oxide is then reduced to metallic copper in a current of hydrogen. We produce, as described and figured in the case of arsenic (§ 471), a powerful current of hydrogen, dry it by a passage through concentrated sulphuric acid, and cause it to traverse the filter-tube by means of a small tube in the caoutchouc stopper, the filter-tube being placed in a slightly inclined position, with its point fixed downwards. After fifteen minutes, when all the atmospheric air has been expelled, the hydrogen issuing from the tube is ignited, and the filter-tube is heated to about 140° by gently passing the gas flame along it. There occurs the reaction: $Cu_2O + H_2 = Cu_2 + H_2O$; the water is separated in small drops in the narrow part of the tube, and must be gradually expelled by cautiously moving the gas flame. When this has been effected, and all the red Cu_2O, along with any black CuO which has been temporarily formed, is converted into metallic copper (change of colour)—for

which five minutes always suffice—it is allowed to become perfectly cold in the current of hydrogen (about fifteen minutes), air is caused to pass through it for a short time, and it is weighed. Previous to the experiment the tube will have been dried in the drying-closet and weighed; the increase of weight gives the quantity of copper.

The calculation of the sugar is effected by means of the following table (Allihn):[1]—

Milligrammes of Copper corresponding to Milligrammes of Grape-Sugar.

Cu.	Sugar.	Cu.	Sugar.	Cu.	Sugar.
10	6·1	160	81·7	310	162·0
20	11·0	170	86·9	320	167·5
30	16·0	180	92·1	330	173·1
40	20·9	190	97·3	340	178·7
50	25·9	200	102·6	350	184·3
60	30·8	210	107·9	360	190·0
70	35·8	220	113·2	370	195·7
80	40·8	230	118·5	380	201·4
90	45·9	240	123·9	390	207·1
100	50·9	250	129·2	400	212·9
110	56·0	260	134·6	410	218·7
120	61·1	270	140·0	420	224·5
130	66·2	280	145·5	430	230·4
140	71·3	290	151·0	440	226·3
150	76·5	300	156·5	450	242·2

Example.—If from 25 *cc.* of wine there were obtained and weighed, in the first determination, 0·120, and in the second 0·118 *grm.* of metallic copper, the mean, 0·119 *grm.* = 119 *mgrm.* Cu, corresponding to 60·6 *mgrm.* dextrose in 25 *cc.* = 0·2424 *grm.* sugar in 100 *cc.* of wine.

Determination of grape-sugar, as well as of other kinds of sugar, may also be effected with the aid of the polarimeter. The general principles of this method may be found in § 29, and some especial details in the chapter on Wine.

§ 220. **Levulose.**—Occurs rarely without the presence of dextrose. It is determined chemically exactly like dextrose. Allihn's table is indeed used for ascertaining how much sugar corresponds to the copper, although the proportions of reduction are not quite identical.

Milk-Sugar, Lactose.—After removing the albumenoids from about 25 *grm.* of milk according to Ritthausen (§ 279), the liquid is filled up to about 500 *cc.* Of the solution of lactose thus obtained, containing

[1] Wein has recently revised Allihn's table by his own special experiments, and has obtained values which deviate merely to an unimportant extent. (*Chemiker Zeitung*, 1890; *Repertorium*, 106.)

about ½ per cent. of milk, 100 *cc.* are added to 50 *cc.* of Fehling's solution in a porcelain capsule holding about 300 *cc.* It is covered with a glass plate, and boiled for six minutes. The Cu_2O is filtered off as directed for dextrose, reduced, and the quantity of sugar is ascertained from the copper by means of the following table (Soxhlet's) :—

Milligrammes of Copper representing Milligrammes of Lactose.

392·7	. .	300	300·8	. .	225	204·0	. .	150
363·6	. .	275	269·6	. .	200	171·4	. .	125
333·0	. .	250	237·5	. .	175	138·3	. .	100

Titration is also possible; for this purpose the solution should contain 1 per cent., so that about 33 *cc.* = 50 *cc.* of Fehling's liquid. Boil for six minutes. 50 *cc.* of Fehling's solution = 0·347 lactose.

Maltose.—To 50 *cc.* of undiluted Fehling's solution add in the cold 25 *cc.* of solution of maltose at from 0·6 to 1 per cent.: boil for four minutes, filter through asbestos, and reduce as described for dextrose.

If maltose has to be determined along with dextrine, we proceed as described at the end of this section. The calculation is effected according to the following table (Wein) :—

Milligrammes of Copper represent Maltose.

30	. .	25·3	130	. .	113·4	220	. .	193·9
40	. .	33·9	140	. .	122·4	230	. .	202·9
50	. .	42·6	150	. .	131·4	240	. .	211·8
60	. .	51·3	160	. .	140·4	250	. .	220·8
70	. .	60·1	170	. .	149·4	260	. .	229·8
80	. .	68·9	180	. .	158·3	270	. .	238·8
90	. .	77·7	190	. .	167·2	280	. .	247·8
100	. .	86·6	200	. .	176·1	290	. .	256·6
110	. .	95·5	210	. .	185·0	300	. .	265·5
120	. .	104·4						

For the determination of maltose in beer the following method may be recommended for a speedy approximation (Reischauer) :—

10 *cc.* of beer are diluted to 100 *cc.*, and 5 *cc.* are put in each of ten test-glasses, which are arranged in a vertical position around a central support by means of a star-shaped holder. To each glass we add a little of Fehling's solution—to the first 0·6 *cc.*, to each following glass 0·1 *cc.* more, and to the last therefore 1·5 *cc.* The entire set is then placed for ten minutes in a water-bath in lively ebullition. On taking the apparatus out, some cuprous oxide is found separated out in each glass. Where an excess of Fehling's solution had been added the supernatant liquid is blue, but where the maltose was in excess it is yellow. We find the glass the colour of which shows no greenish tint (in case of need we must take the mean of the contents of two glasses), and the proportion of maltose is obtained by a single calculation, as 0·1 *cc.* of Fehling's solution reduces exactly 0·00075 of maltose. The calculation is carried out in the following table :—

Cc. of Fehling's Solution.	Grm. Maltose in 100 cc.	Cc. of Fehling's Solution.	Grm. Maltose in 100 cc.
0·6	0·900	1·10	1·650
0·65	0·975	1·15	1·725
0·7	1·050	1·20	1·800
0·75	1·125	1·25	1·875
0·8	1·200	1·3	1·950
0·85	1·275	1·35	2·025
0·9	1·350	1·4	2·100
0·95	1·425	1·45	2·175
1·0	1·500	1·5	2·250
1·05	1·575		

Example.—If the colour at 1·20 *cc.* is green, at 1·10 *cc.* yellowish green, and at 1·00 distinctly yellow, 1·05 is the quantity of Fehling's solution reduced, and there are consequently 1·575 *grm.* maltose in 100 *cc.* of beer.

Cane-Sugar.—The process of inversion (conversion into invert-sugar), according to the formula—

$$\underset{\text{Cane-sugar.}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Dextrose.}}{C_6H_{12}O_6} + \underset{\text{Levulose.}}{C_6H_{12}O_6}$$

is effected by different methods.

Clerget heats 100 *cc.* solution of sugar for fifteen minutes to 60° or 70° on the water-bath along with 10 *cc.* of concentrated pure hydrochloric acid. Soxhlet and Meissl heat 9·5 *grm.* cane-sugar with 700 *grm.* water and 100 *cc.* of one-fifth normal hydrochloric acid to 100° for thirty minutes in the water-bath, neutralise with a standardised soda-lye, and make up to 1 litre. Gabbe recommends oxalic acid as the best inverting agent. The neutralised solutions are worked up exactly like solutions of dextrose; the calculation of the weighed copper is effected according to the following table (Meissl):—

Milligrammes of Copper corresponl to Milligrammes Invert-Sugar.

96·0	50	318·9	175
142·9	75	360·3	200
188·9	100	400·1	225
233·2	125	428·1	245
276·8	150		

100 *grm.* invert sugar = 95·55 *grm.* cane-sugar.

Dextrine.—Dextrine is more difficult to invert; its determination is required almost exclusively in beer. We heat 40 *grm.* beer with 6 *cc.*

of dilute sulphuric acid (1 : 5) in a sealed tube or a pressure-flask for six hours in a sodium chloride bath at from 108° to 110°. At the expiration of this time all the dextrine, and also all the maltose, are converted into grape-sugar, and after neutralisation with soda and dilution to 100 *cc.*, the filtrate is treated exactly as directed for dextrose. In another unaltered specimen of the beer the maltose is determined. The calculation is best shown by an example: 10 *grm.* beer yielded direct 0·0751 Cu, *i.e.*, in 100 *grm.* 0·66 *grm.* maltose.

Of the inverted 40 *grm.*, which were diluted to 100 *cc.*, yielded 10 *cc.* (= 4 *grm.* beer), 0·1728 *grm.* Cu corresponding to 0·0884 dextrose. Hence in 100 *grm.* inverted beer there are 2·21 *grm.* dextrose. 9·5 parts maltose yield 10 parts of dextrose, and these 10 parts of dextrose correspond again to 9 parts of dextrine. The 0·66 per cent. of maltose, directly determined in beer, have therefore, on inversion, been transformed into 0·695 per cent. of dextrose. If this is deducted from the total grape-sugar there remains 2·21 − 0·695 = 1·515 per cent. of dextrose, which corresponds to 1·363 per cent. of dextrine.

§ 221. **Starch.**—If cellulose is not simultaneously present, the starch is freed from sugars and dextrine by extraction with cold water, and the residue is boiled with abundance of 10 per cent. hydrochloric acid (100 *cc.* to 1 *grm.* starch), boiling in a roomy flask on the water-bath for four hours. All the starch is converted into grape-sugar, which is determined as above; ten parts of dextrose found represent nine parts of starch.

$$\text{Grape-sugar} = \underset{180}{C_6H_{12}O_6} : \text{starch} = \underset{162}{C_6H_{10}O_5} = 10 : 9.$$

If cellulose is present, the figures obtained by the method described are rather too high, since a little of the cellulose is converted into sugar when boiled with the hydrochloric acid; but in many cases this fact is ignored in analyses. The error may be avoided if the starch, made into paste by heating, is treated with extract of malt.[1]

We heat from 1 to 2 *grm.* of the starch-paste with 15 *cc.* extract of malt to from 40° to 50° in the water-bath for one hour, and then allow it to stand for twenty-four hours,

[1] *Extract of Malt.*—25 *grm.* of new malt are crushed and heated with 250 *cc.* of water for one to two hours at from 30° to 40° in the incubation-closet, and the extract is filtered off. It contains a little dextrine and sugar, which must be especially determined in a specimen after inversion.

preferably in the incubation-closet. The starch is then converted into dextrine and maltose, which may be removed from the cellulose by means of water, and then converted into dextrose by boiling with an acid. The sugar found in 15 *cc.* of the inverted extract of malt is deducted. If we use 0·05 to 0·1 diastase (Lintner) instead of extract of malt, we dispense with the examination of the latter.

If an autoclave is available the starch may be determined in the following manner without attacking the cellulose. From 3 *grm.* of the substance we extract the sugar and the dextrine with cold water; the substance is then made into a paste with 100 *cc.* of water in bottles containing 150 *cc.*, which are then heated in the autoclave for three hours at a pressure of three atmospheres. The hot solution now contains all the starch in a soluble modification, which can easily be filtered off through asbestos. When we are satisfied after washing with water that particles of the residue do not show a blue coloration under the microscope, the starch is converted into sugar by boiling with dilute hydrochloric acid as directed at the beginning of § 221.

Cellulose is determined by difference: *e.g.*, subtracting from 1 *grm.* the quantities, determined in 1 *grm.*, of water, ash, inorganic acids, fatty substances, albumenoids, sugar, and starch. This result is not very accurate, as the errors readily accumulate. Or we may proceed, according to the method of Weende, for determining crude fibre, as follows: 3·5 *grm.* of the substance are boiled firstly for thirty minutes with 200 *cc.* of sulphuric acid at 1¼ per cent., then twice with water, then for thirty minutes with 200 *cc.* of soda-lye at 1¼ per cent., and then again twice with water. Before each change of liquid the mixture is allowed to subside and decanted off cautiously only as far as it can be poured clear. Finally, the residue of cellulose is filtered upon a weighed filter. After washing and drying it is weighed again, and finally incinerated. The weight of the filter + cellulose − weight of filter-ash, gives the weight of cellulose. This method also is not very accurate, since a little cellulose is always dissolved. Or, finally, we may convert the cellulose into grape-

sugar by very prolonged boiling with sulphuric acid in pressure-flasks, determine it as such, and calculate it like starch.

We may add a few supplemental remarks on the detection of several carbo-hydrates when they occur jointly.

Cellulose, starch, and dextrine may easily be separated from the other carbohydrates according to what has been said (§§ 215 and 216), or they may be determined in presence of a directly reductive kind of sugar. Cane-sugar does not affect the determination of the directly reductive sugars. If cane-sugar has to be determined along with dextrose or levulose (in fruits and in wines, or honey mixed with cane-sugar), we determine firstly in one portion the dextrose or levulose, and then invert a second portion, and boil it with Fehling's solution ;theincrease of copper is to be referred to invert-sugar, and calculatedas cane-sugar

In the same manner milk-sugar may easily be determined in presence of cane-sugar (condensed milk) ; on the inversion of the cane-sugar the milk-sugar (lactose) is also resolved into galactose and dextrose, which mixture reduces like invert-sugar. We calculate how much invert-sugar the milk-sugar is capable of yielding (95·55 *grm.* milk-sugar = 100 *grm.* invert-sugar), and deduct this quantity from the total quantity of invert-sugar which has been found. The residue of invert-sugar we calculate as cane-sugar. If, on the other hand, several directly reductive sugars are simultaneously present, *e.g.*, levulose or lactose along with dextrose (in fruits or confectionery), we are generally content in practice to refer the total sugar to the kind which is probably most abundant.

B. Examination of the Utilisable Character of Foods.

§ 222. It is well known that the question is not merely what nutrient substances are consumed in any article of food, but how large a proportion of them is absorbed in the digestive organs. For determining the utilisability, *e.g.*, of meat,[1] we proceed as follows :—

A healthy person is allowed to consume (according to Voit and Rubner) :—

On the first day, a mixed diet.

[1] If it is required to determine the utilisation of vegetable food, meat is generally employed for marking out the "boundary" of the experimental solid excrement. It is convenient to take, along with the meat, black-pudding, in order to obtain very dark fæces. (Powdered charcoal and bilberries have also been taken on the days before and after the days of the experiment, in order to colour the "boundary" fæces. Personally I have not seen very desirable results. This procedure is much praised by others, but in any case such colouring-agents must be well mixed and stirred up with a more moderate "boundary" agent, *e.g.*, thick barley gruel, &c.)

On the second day, only 2 litres of milk in three portions, the latest being taken not after 4 P.M.

On the third day we begin with the use of meat at 8 or 10 A.M. In the course of the day there are taken from 2 to 3 lbs. of meat, prepared in the manner to be examined.

On the fourth day, the same as on the third.

On the fifth day, nothing is taken until noon, then from ½ to 1 litre of milk. In the evening, again, about 1 litre of milk.

On the sixth day, a mixed diet.

During the milk and meat days no other solid food must be taken: at most a little cheese, finely masticated, may be taken on the milk days; on the meat days, a bottle of white wine, a little seltzer water, or from 1 to 1½ litre beer is permissible for persons who are habituated to them. Of condiments, salt and pepper are allowed according to taste, and, if desired, small quantities of other flavourings.

From the first milk day onward the fæces must be carefully voided upon movable porcelain plates, suitably placed. The yellowish white, very characteristic milk fæces are sharply distinguished from the dark brown meat fæces. Any trifling intermixtures at the ends may generally be separated from each other with sufficient accuracy by means of careful manipulation with a horn spatula. The several portions of the fæces corresponding to the meat period are each time weighed whilst recent, spread out united in a thin layer on a porcelain plate, and rendered air-dry[1] at a gentle heat, carefully scraped off the plate, and weighed again. It is then powdered as finely as possible, and we determine in portions of

2 to 3 *grm.* moisture and ash,

6 to 8 *grm.* the ethereal extract (mostly calculated as fat),

1 *grm.* the nitrogen by Kjeldahl.

From the numbers obtained we calculate how much dry matter, how much ash, how much fat and nitrogen go to waste in the fæces, or, in other words, what percentage of

[1] If the fæces are strongly alkaline, 1 *grm.* tartaric acid, dissolved in a little water, is to be stirred in, to prevent loss of ammonia on drying: the tartaric acid is of course to be taken into account in calculating the results.

the constituents of the food ingested reappear unutilised in the fæces. If cellulose is in question, it must be examined as in § 221 (see also Rubner, *Zeitschrift f. Biologie*, 1883).

Along with this calculation must be made an analogous examination of the food ingested, using carefully selected average specimens. In case of meat, the entire supply for the two days should be chopped up, and specimens for analysis are taken after being carefully mixed up. Bread, vegetables, &c., should be placed in readiness at the beginning of the experiment.

§ 223. What has been said contains only the main outlines of the arrangement of the experiments. For carrying out such investigations for hygienic purposes, and for a correct appreciation of the results, the following points must further be considered:—

1. A man, even if fasting, or if consuming non-nitrogenous food, perfectly absorbable, excretes daily a quantity of 13·4 *grm.* dry fæces, with 0·73 *grm.* nitrogen. These quantities are therefore to be deducted from the total dry substance, or respectively from the nitrogen, as determined. See Rieder (*Zeitschrift f. Biologie*, 1884). This does not take place in the ordinary statements, as, *e.g.*, in the accompanying table by Prausnitz (results obtained chiefly by Rubner, partly by Prausnitz and others).

On the Consumption of	There are Wasted per Cent. of the Ingested		
	Dry Substance.	Nitrogen.	Ash.
Rice	4·1	20·4	15·0
White bread	4·4	22·2	21·3
Rusks	4·9	20·5	20·9
Maccaroni	5·0	14·1	23·1
Meat	5·1	2·6	18·1
Eggs	5·2	2·6	18·1
Maize	6·7	15·5	30·0
Milk (by children)	5·7–6·7	4·4	42·8
Milk (by adults)	8·5–9·0	8·3–11·2	37·1–47·1
Potatoes	9·4	32·2	15·8
Black bread	15·0	32·0	36·0
Brocoli	14·9	18·5	19·3
Carrots	20·7	39·0	33·8
Pease pudding	9·1	17·5	32·5
Beans (boiled soft, not crushed)	18·3	30·2	28·3
Mixed diet	8–12	...	...

If we take Rieder's figures into account, meat is as good as completely utilised.

2. It must be remembered that the utilisation of food varies according to the cooking, the manner of mastication, &c. Vegetable albumen especially is properly utilised only if the seeds are finely comminuted and boiled soft. As an instance we may point out in the above table the very different utilisation of pease-pudding and of beans boiled soft but not crushed. A fine pulp of beans will certainly be as well utilised as the pulp of pease.

3. Lastly, mixed foods seem to be utilised differently from, and generally better, than single unmixed ingredients. Thus, Rubner observed that milk with the addition of cheese (*Zeitschrift f. Biologie*, xv. 139), and Malfatti that maize-meal with the addition of cheese were much better utilised than milk or maize-meal alone. (*Sitzungsberichte der Wiener Akad.*, 1884, vol. cx., part iii., December part.)

The term, "easily digestible," is used with such varying and ill-defined connotation by the lay public (*e.g.*, in part as completely, in part as quickly- and in part as painlessly digestible), that it is preferably abandoned in scientific writings, and that we say instead :

1. A food is well or badly utilised.
2. A food remains in the stomach for a long or a short time.
3. A food is slowly or rapidly absorbed.
4. A food during digestion occasions unpleasant sensations, or it does not. The notion of easy or difficult digestion is mostly used by the public in this sense. Voit proposes to use instead "easily tolerated."

C. Decision on the Nutritive Value and Nutritive Cost of Articles of Food.

§ 224. In considering the nutritive value of foods we ask: —How much albumen, fatty matter, and assimilable carbohydrates, does it contain ? Water, cellulose, which is utilisable only to a trifling extent, and salts, are not considered.

As 100 *grm.* fat during combustion, whether within or without the body, yield as much heat as 213 *grm.* albumen or 240 *grm.* carbohydrates (Rubner), or, in other words, are isodynamic, we might express the relative nutrient value of the substances as follows: If we have in 100 *g.* substance, *a.g.* albumen, *b.g.* fat, and *c.g.* carbohydrates, the relative nutritive value is: $a \cdot \frac{100}{213} + b + c \cdot \frac{100}{240}$. The values thus obtained, however, scarcely give values which are practically useful, since the several nutritive substances are no perfect substitutes for each other, as, *e.g.*, fats and carbohydrates cannot supply the nitrogen expended by the body.

Our foods are in fact by no means valued according to the calorific value just calculated. The albumenoid bodies, relatively difficult to procure and yet indispensable, are valued more highly than the isodynamic quantity of carbohydrates. The agreeable taste, and the difficulty of procuring, play a decisive part for the valuation of our foods. It has been sought, by the most varied methods, to obtain an insight into the proportion of market-prices to the true nutritive values. After what has been said above, we need not wonder that all these considerations are open to various objections; the simple method indicated by Emmerling, and since adopted by König, seems to me to correspond fairly well with actual relation. We assume

1 *grm.* carbohydrate has the value of one nutrient unit.

1 *grm.* fat has the value of three nutrient units.

1 *grm.* albumen has the value of five nutrient units.

It must again be brought into prominence that these "nutrient units" do not represent a physiological but merely a national economic standard.

We ascertain how many nutrient units are contained in 100 *grm.* of a substance: the more such units it contains the greater is its value. Further, we may directly calculate how many nutrient units we obtain in any case for one shilling?

No attention is here given to the varying utilisability of the several foods; the method can in itself yield no accurate values, so that the introduction of utilisation would merely complicate the method without introducing much improvement.

Example according to König:—

	Water per Cent.	Albumen per Cent.	Fat per Cent.	Nitrogen from Extraction Matter.	Ash.	Sum of Nutrient Units per Kilo.	Price per Kilo.[1]	1000 Nutrient Units Cost.	Obtained for One Shilling.
1. Mutton, very fat	47·91	14·80	36·39	0·05	0·85	1832·2	148	80·8	1238
2. Sheep's tongue .	67·44	14·29	17·81	0·09	1·00	1230·8	183	148·6	672
3. Sheep's liver . .	69·30	21·64	4·98	2·73	1·35	1258·7	85	67·5	1481

[1] 1832·2 is the value found: $(14{\cdot}80 \times 5 + 36{\cdot}39 \times 3 + 0{\cdot}05) \times 10$.

As the utilisation of meat and tongue will not differ, and the liver (at least by dogs) is utilised nearly as well as the meat (Bergeat), it is advisable, if we consult simply physiology and market prices, not to buy tongue, but with most advantage liver, if the choice lies among these three substances.

Some further values, calculated according to this method, are given in the subjoined table by König.

	Nutrient Units per Kilo.	Market Price per Kilo (1878–80).	1000 Nutrient Units Cost.	For One Shilling we Obtain Nutrient Units.
Skim milk	216	9·0	41·7	2400
Lean cheese	1914	82·7	43·2	2314
Milk	320	15·0	46·8	2133
Bacon	2767	172·0	62·1	1608
Pork	1836	131·0	71·4	1401
Semi-fat cheese	1970	141·7	71·9	1319
Butter	2610	213·3	81·7	1223
Veal	1157	112·0	96·8	1033
Beef	1168	128·3	109·8	911
Beans	1755	22·5	12·8	7800
Pease	1713	28·9	16·8	5927
Lentils	1842	37·0	20·1	4979
Potatoes	304	6·1	20·1	4982
Rye flour	1328	31·3	23·5	4243
Wheat flour	1328	38·7	29·1	3431
Rice	1177	58·0	49·3	2029

D. Some Hints for an Examination of and a Decision upon Dietary Scales.

§ 225. If the question is to decide on the maintenance of a person from a hygienic point of view we must determine:—

1. On at least three successive days the quantities which the person consumes of each single article of food. For this purpose each food is weighed before the meal as well as the residue left in the dish.

2. For a useful survey of the composition of food an analysis is generally unnecessary, except in case of quite unusual conditions (peculiar national dishes), &c. Our simple native dishes have been so often analysed that it generally suffices to use the mean values given by König, and calculated from the appended tables. If the expert intends to

analyse personally (which is indispensable for *accurate* results), the utmost care must be used in taking large, thoroughly mixed average samples, which, after they have served for the determination of moisture, must be pulverised as finely as possible.

If the diet of numerous persons has to be examined, *e.g.*, that of the inmates of a prison, or of barracks, we may proceed in two manners:—

1. We satisfy ourselves by calculating how many grammes albumen, fat, and carbohydrates were present in the articles of food served out according to the kitchen account-book during a long time, and divide this total by the number of days multiplied by the number of persons fed. In this manner we obtain scarcely a correct survey, much less accurate figures.

2. We take the more laborious process of weighing daily how much of every kind of food is given out from the kitchen, and how much comes back unconsumed; the difference is divided by the exact number, carefully ascertained, of the persons fed. In this manner we avoid the error of calculating as nutrient substances the waste (bones, husks, shells, &c.), and obtain a very different insight into the state of the case, which, according to 1, often appears much too favourable. Analyses are here not absolutely necessary, but they are indispensable for bread, porridge, gruels, and other moist foods, which are prepared differently in different districts. The proportion of nitrogen in bread and vegetables fluctuates very strikingly according to the season and the variety. See Forster, *Massenernährung in Kriegs und Friedenszeiten* (*Münch. Med. Wochenschrift*, 1890, p. 637). The memoir refers also to Rosenheim (*Pflüger's Archiv*, 1890). If the ingestion of albumen is insufficient, the digestion of fatty matter is disturbed possibly by a decreased formation of ferments.

In deciding upon a dietetic scale, we have to distinguish the following points:—

1. Is the diet sufficient? Voit demands for a healthy adult as normal diet 118 *grm.* albumen, 56 *grm.* fat, and 500

grm. carbohydrates, on the supposition that medium work has to be performed. Of the albumen, if possible one-third, *i.e.*, 38 *grm.*, corresponding to 190 *grm.* pure meat, or 1 litre milk, or 125 *grm.* cheese, or five eggs, should be ingested as animal albumen, the remaining two-thirds as vegetable albumen. This prescription is not founded on the consideration that animal albumen, as such, is utilised by the body differently from vegetable albumen, but it is better utilised, renders the food less bulky, and is generally present along with enjoyable substances. Temporarily the proportion of albumen in the diet can be reduced without injury (70 to 40 *grm.* albumen), but the assertion that 70 *grm.* albumen are permanently sufficient is scarcely sufficiently demonstrated. See Forster, *Massenernährung in Kriegs- und Friedenszeiten* (*Münch. Med. Wochenschrift*, 1890, p. 637). The writer refers to Rosenheim (*Pflüger's Archiv*, 1890), who observed that if the ingestion of albumen is insufficient the digestion of fatty matter suffers. See also Munk and Rosenheim, *Ueber den gesundheitsschädigenden Einfluss des fortgesetzten Genusses eiweissarmer Nahrung* (*Hyg. Rundschau*, 1891, p. 524). It must never be forgotten that the inmates of such institutions as prisons are less easily kept at a sufficient condition of strength than men living at large, perhaps in consequence of the want of exercise in the open air, and the absence of articles of enjoyment of their own selection. Consequently they cannot be satisfactorily nourished with the lowest scale which suffices for persons at liberty.

It is advantageous to increase the fat at the expense of the carbohydrates, giving, *e.g.*, 90 *grm.* fat and 410 *grm.* carbohydrates. As ordinary vegetables supply only about 25 *grm.* fat, we should add, as a minimum, the fat (30 to 35 *grm.*) of 1 litre milk, or 125 *grm.* fat cheese; it is still better, as said, to add also from 30 to 40 *grm.* of fat as butter, bacon, &c.

2. Are the articles of food, above all is the meat, sufficiently boiled or roasted (any parasites present being killed), and are the vegetables, especially potatoes, peas, beans, &c., boiled soft? By cooking starch granules are converted into

gelatinised starch, gelatigenous tissues converted into gelatine, cellular membranes burst, cellular connections relaxed, and parasites, animal or vegetable, are killed.

3. Is variety introduced into the diet by the suitable use of seasonings? Especially sauerkraut, cucumbers, sour milk, herrings, cheese, smoked and salted meat, are articles of food which affect the diet most favourably by the presence of substances which give enjoyment.

4. Is not the diet too exclusively confined to the state of porridge, or is it varied in its consistence? The constant use of semi-fluid food produces nausea.

5. Does it present too bulky or too small a volume? An adult, besides the ordinary drinks, requires daily about 1600 to 1800 *grm.* of food if he is to feel satisfied. Prisoners generally receive from 3100 to 3900 *grm.* on account of the excessive moisture of the diet, and the too abundant supply of carbohydrates.

6. Is the dietary composed of appetising, sound constituents, such as agree with the consumer? See here the special chapters on foods.

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SECTION V.

CHEMICAL AGENTS FOR THE PRESERVATION OF FOODS.

A. Examination for Chemical Preservative Agents.

§ 226. It seems convenient to give here, in a general manner, the examination for chemical preservative agents, and refer to them subsequently when discussing the several articles of food. We have to consider:

Boric acid and boric preparations.

Sulphurous acid and sulphites.

Benzoic acid, salicylic acid, and in conjunction with them the "preservative salts," consisting of the most varied mixtures of these agents, with sodium chloride, soda, saltpetre, &c.

Common salt, carbonic acid, and vinegar do not require special discussion from this point of view, although they have a preservative action. Their presence generally betrays itself at once to the senses, and they are toxicologically important only because they may dissolve metals from the vessels in which preserved foods are enclosed.

1. Boric Acid and Preparations of Borax.

§ 227. Formerly boric acid (BO_3H_3) and borax ($Na_2B_4O_7 + 10H_2O$) were more particularly employed, but recently other less known boric preparations have come into use. All these preparations have a very slight taste, and are readily soluble in water.

Sodium Chloro-borosum is said to be sodium borate mixed with chloride. It is alleged to be prepared from boric acid, sodium borate, and common salt. It always gives off a little free chlorine.

Barmenite is a preparation recommended for preserving meat, and said to contain a "certain percentage of sodium chloro-borate." According to C. Schwarz it consists of: 80 borax, 15 boric acid, 3 NaCl, 1 sodium chlorate, and traces of alumina.

Boroglyceride is a vitreous mass, tasteless, and inodorous, obtained by melting together 62 parts boric acid and 92 parts glycerine.

Qualitative Detection (Meissl). The substance is rendered alkaline with milk of lime, evaporated to dryness, and incinerated. The ash is dissolved in the smallest possible quantity of concentrated hydrochloric acid, separated from carbonaceous matter by filtration, and the filtrate is evaporated to dryness on the water-bath. An appreciable loss of boric acid is not to be apprehended. The mass is then moistened with hydrochloric acid, slightly diluted, saturated with tincture of turmeric, and evaporated to dryness on the water-bath.

In presence of the slightest trace of boric acid the dry residue appears of a scarlet or cherry colour. The reaction is extremely sensitive. 1 *mgrm.* to 0·5 *mgrm.* of boric acid in the ash, or, *e.g.*, 0·001 to 0·002 per cent., may be determined in milk in this manner with the greatest certainty. Errors are not easily possible.

Concentrated hydrochloric acid indeed gives a cherry-red colour, with tincture of turmeric, but it at once disappears on the addition of water, and turns brown on drying; whilst the boric coloration only appears on drying, and is afterwards removed by water only if boiling, or used in excess.

Turmeric paper gives the same colour with both acids. The red colour adheres very obstinately to the vessels, but it may be readily removed by means of alcohol. Of course the ash thus tested with turmeric may be further used for the flame reaction, by introducing a small portion moistened with hydrochloric acid into a spirit-flame upon a platinum wire. The flame has a green margin.

The *quantitative determination* presents great difficulties, especially in presence of sodium salts, and can be effected only by experienced chemists. Traces of boric acid are very widely distributed in nature (carrots, beet-root sugar, Californian wines, &c.), and are often contained in the glaze

of many vessels (Kayser), therefore caution! According to Venable and Collison the caustic alkalies of the best firms regularly contain boric acid (0·1 per cent.). *Chemiker Zeitung*, 1890. *Repertorium*, p. 117.

2. Sulphurous Acid. Sulphites.

§ 228. The forms of this acid in most general use are: 1. Gaseous sulphurous acid (SO_2). 2. Acid or primary calcium sulphite (acid sulphite of lime) $(SO_3H)_2Ca$, which can exist only in aqueous solution, whilst neutral or secondary calcium sulphite, SO_3Ca, is sparingly soluble in water. 3. Acid potassium and sodium sulphite, SO_3HK, a crystalline substance of an acid taste readily soluble in water.

Qualitative Detection.—The intense odour of sulphurous acid (SO_2), especially after acidulation with phosphoric acid, is perceptible only if it has been used very boldly for the preservation of articles of food. If only small quantities are present, the following preliminary test is applied: The beer or wine to be examined is mixed with hydrochloric acid and zinc, and there is laid upon the test-tube a slip of filter-paper moistened with basic lead acetate. If a brown or black colour rapidly appears ($SO_2 + 3\,H_2 = SH_2 + 2\,H_2O$), we must verify if SO_2 was really present; if the slip of paper does not become coloured, we may be certain that no sulphurous acid was present. According to recent investigations, sulphurous acid may appear in various fermentations, whether from the reduction of sulphates or from albumenoid substances, and the mere qualitative recognition of sulphurous acid, or its salts, does not prove that they have been intentionally added as preservative agents. It must be remembered that sulphured hops, the use of which cannot be objected to either hygienically or legally, seem to give off small quantities of sulphurous acid to the wort.

For a *quantitative determination* we add to 200 *cc.* of beer or wine 5 *cc.* phosphoric acid, and distil off 100 *cc.* with a Liebig refrigerator, the condensation-tube of which is drawn

out to a point, and plunges into 20 *cc.* of decinormal solution of iodine (see § 135). It is advisable to conduct this process whilst a current of carbonic acid, previously washed with water, is caused to pass without interruption into the retort. This precaution prevents the reflux of the distillate when the retort cools. The solution of iodine must not be completely decolorised, otherwise the experiment must be repeated with a larger quantity of iodine. The latter converts the sulphurous acid liberated by the phosphoric acid into sulphuric acid ($SO_2 + 2\,H_2O + I_2 = H_2SO_4 + 2\,HI$), which after acidulation with HCl is precipitated in the distillate by means of barium chloride and weighed as barium sulphate (§ 176). The filter is coloured at first blue by free iodine, which, however, does not interfere. 1 *mgrm.* $BaSO_4$ represents 0·2748 *mgrm.* SO_2.

3. Salicylic Acid.

§ 229. *Salicylic Acid.*—$C_6H_4 < \begin{matrix} COOH \\ OH \end{matrix}$ (ortho-oxybenzoic acid) forms well crystallised white needles sparingly soluble in cold water (1 *grm.* in 400 water), very readily in alcohol. Taste sweetish, strongly irritating.

Qualitative Recognition.—If salicylic acid is present in quantity it is easily detected; 50 *cc.* of the liquid, beer or wine, being acidulated with a little sulphuric acid, shaken up in a stoppered cylinder with 50 *cc.* of a mixture of equal parts ether and petroleum-ether, and the clear ethereal extract is drawn off with a syphon and filtered. Pinette recommends in case of beer the addition of 5 *cc.* alcohol before adding the mixture of ether and petroleum-ether to prevent the formation of an emulsion (*Chemiker Zeitung*, 1890, p. 1571).

In the filtrate the ether and the petroleum-ether are completely expelled, and to the small quantity of residual water there are added a few drops of solution of ferric chloride, neutral and very dilute. A violet colour indicates salicylic acid; the intensity of the colour permits a conclusion as to the quantity.

If mere traces of salicylic acid are present in wine or beer (up to $\frac{1}{10}$ *mgrm.* per litre) the ether is not entirely driven off, but only down to about 5 *cc.*; from 3 to 4 *cc.* of water are then added, and a little very dilute solution of ferric chloride. The dirty-coloured liquid is then passed through a filter which has been previously moistened with water. The watery filtrate obtained is violet, whilst the ethereal liquid, of a yellowish or yellow colour, remains in the filter (Röse). For wine this modification had better not be used, since affirmative results are given by wines to which no salicylic acid has been added. Medicus and Immerheiser have demonstrated traces of salicylic acid (or of a substance which yields the same reactions) in pure wines and in lyes of wine.

In case of milk we proceed according to Girard as follows: 100 *cc.* milk and the same volume of water at 60° are precipitated with eight drops of acetic acid and eight drops of a solution of mercuric nitrate, shaken up and filtered. The filtrate is then shaken out with 50 *cc.* of ether, which takes up the salicylic acid. In case of butter the specimen is first extracted with sodium carbonate in water, kneading it thoroughly and then proceeding as in wine.

4. Benzoic Acid.

§ 230. *Benzoic Acid* C_6H_5—COOH. — Properties very similar to salicylic acid. Sodium benzoate is recommended for milk, and said to be about one-third more efficacious.

Qualitative Recognition according to Meissl.—The substance in question (milk with the addition of sea-sand) is evaporated down upon the water-bath with the addition of a little baryta-water, and with frequent stirring. The powder is then acidulated with sulphuric acid and shaken out three or four times with cold alcohol at about 50 per cent. For the separation of lactose and salts it is mixed with a little baryta-water and concentrated, acidulated with sulphuric acid; the benzoic acid is extracted with ether, and obtained in crystals by the evaporation at the temperature of a room, or not exceeding 60°. It may be sublimed upon the water-bath between two watch-glasses, when the space becomes filled with glittering crystalline leaflets. Benzoic acid dissolved in water gives, with a dilute neutral solution of ferric chloride, a fine reddish-yellow colour; but acetic and butyric acids yield an analogous reaction if similarly treated. A quantitative method is wanting.

B. Decision upon Preservative Agents.

§ 231. All effectual chemical preservative agents at certain degrees of concentration injure not merely the micro organisms which excite fermentation and putrefaction, but also human beings. Whilst there exists no doubt on this point,

it is very difficult to lay down exactly the degree of unwholesomeness. Strictly speaking, before use of a preservative agent can be sanctioned, proofs are to be demanded that the substance in question has, on prolonged use, no injurious effects in the proportions which may possibly be used for preserving articles of food, and especially—

1. Our subjective well-being must not be disturbed.

2. No interference with the functions of the body must be demonstrated. The action of the heart, the respiration, innervation, &c., must remain unmodified.

3. The utilisation of food must not be disturbed.

4. Lastly, we must also demand—especially in case of agents which may be used for preserving the food of children and invalids (*e.g.*, milk)—that they shall not endanger the less resistant organisms of young or sickly individuals. The substance should also be harmless for domestic animals.

5. It is finally extremely desirable that every preservative agent which is not absolutely harmless in large doses should be capable of ready detection and quantitative determination, so that the dealer may be at all times kept under control.

We will examine how the several preservative agents fulfil these conditions:—

§ 232. *Boric Acid.*—According to Lazarus (*Zeit. f. Hygiene*, viii.) milk can only be preserved by means of boric acid if the quantity added exceeds that which can be used without altering the taste. The microbicide action of boric acid in milk is indeed very feeble. From 1 to 2 *grm.* per litre is without appreciable action. How much boric acid enters into meat which is sprinkled over, or is soaked in a solution of boric acid, &c., is hitherto not accurately known, but according to the statement of a patentee, Roosen, who uses a 2 per cent. solution of boric acid under pressure, 500 *grm.* of meat take up only ½ *grm.* boric acid. Covering meat with a 1 per cent. solution of boric acid increases its durability from four to seven days.

Subjective phenomena are generally absent after the ingestion of 1, 2, or 3 *grm.* in very dilute solutions; concentrated solutions are much less

readily tolerated. 2 *grm.* dissolved in 50 *grm.* water occasioned Mattern violent pain in the stomach and diarrhœa. Rabbits and dogs when drenched daily with doses of ½ *grm.*, or 1 to 2 *grm.* boric acid in 20 or 50 *grm.* water, became in a few days unwell, and suffered from diarrhœa, salivation, and emaciation. In some experiments a fatal result was observed, but such cases have hitherto been not very numerous. On several occasions severe illness is recorded in human subjects on washing out the stomach and the bladder, when some grammes of boric acid were left behind dissolved in relatively small quantities of water, as also on subcutaneous injection.

The assertions of Artmini and Polli (*Annali di Chimica Applicata alla Medicina*, 1877) that doses of 3 to 4 *grm.* continued for months were well tolerated by men, are characterised by Mattern as incredible. These observations may, however, be reconciled with those of Mattern. Mattern has, in general, caused to be swallowed concentrated solutions, or even crystals of boric acid, which would cause irritation not encountered in case of dilute solutions. But if the results of Mattern have fallen out rather too unfavourably, the researches of Forster testify very distinctly against boric acid.

Forster and Schlenker have shown in very careful and repeated experiments on the utilisation of food in human subjects that a daily dose of from ½ *grm.* to 3 *grm.* of boric acid (BO_3H_3) added to human diet affects the absorption of the nutritive substances ingested, and probably occasions an increased separation of intestinal epithelia, or an increased secretion of intestinal mucus.

Borax, according to Forster, will probably act like boric acid; concerning "natrium chloro-borosum," barmenite, &c., experiments on man are wanting, and for the present they must be placed on the same level as boric acid and its mixtures. Their use for the preservation of meat is said to be greatly extending, but preparations of borax for preserved meats are prohibited for the German navy.

Liebreich (*Berlin Klin. Wochenschrift*, 1887) has advocated the harmlessness of the quantities of boric acid required for preservative uses, but without bringing forward any new experiments.

§ 233. *Sulphurous Acid.*—Hitherto this gas has been regarded as almost indispensable in the production of wine; it serves to destroy the micro-organisms which establish themselves on the inner side of the casks. In a 12-hectolitre cask there are burnt at each sulphuring 20 to 30 *grm.* sulphur, and the wine takes up more or less of it, according to the method of procedure. Besides killing the microbia on the inside of the staves, sulphurous acid regulates the fermentation, renders young wines fit for bottling, and causes older wines to assume very gradually the flavour of old wine, &c.

Further. SO_2 is one of the best remedies for the diseases of wine (see Wine). In beer the reason for its use is similar; the beer keeps better, after fermentations are checked. Here the solutions of calcium bisulphite with which the casks and the wash-tubs, &c., are rinsed out, and the cellars are coated, play a part. The vessels must always be subsequently rinsed out with water.

Whilst at present no one objects to traces of SO_2 in wine or beer, it is without doubt that large quantities, as they are used as an addition to beer for a fraudulent purpose (also in the form of calcium bisulphite), are injurious to health. But where the boundary lies is not yet accurately known, in spite of the careful researches of L. Pfeiffer, Munich.

Pfeiffer, with good reason, entertains the opinion that published statements which seem to show the harmlessness (Polli) of sulphites to the extent of 8 to 12 *grms.* must refer to preparations which have lost much of their poisonous properties by conversion into sulphates. Bernatzik and Braun have met with quite different results in numerous unobjectionable experiments.

Thus 80 *mgrm.* of free sulphurous acid dissolved in sugar-water and distributed over twenty-four hours, were badly tolerated by the majority of the persons experimented upon (women lying in); the consequences were severe diarrhœa, vomiting, and discomfort lasting for days; even doses of 1 *grm.* magnesium sulphite containing 0·3 *grm.* SO_2 were for the most part badly tolerated, exciting vomiting and diarrhœa. Still one-third of the patients who received daily 3·75 *grm.* $NaHSO_3$ (containing 2·28 *grm.* SO_2), and two-thirds of those who received 3·75 *grm.* $KHSO_3$ (containing 1·98 *grm.* SO_2), experienced no remarkable inconvenience; the medication was tolerated. The other women were attacked with disturbance of the bowels.

Pfeiffer himself took, along with several friends, 0·5 $NaSO_3$ strongly diluted (*i.e.*, 250 *mgrm.* SO_2); the consequences were pressure and pain in the stomach, general discomfort and eructations. In my laboratory doses of 100 *mgrm.* have been many times taken without injury.

Unfortunately Pfeiffer did not make the experiments of the greatest hygienic importance, the ingestion of small doses of the salt (sulphite) or the acid in a very dilute state during a long time, and by healthy men. Pfeiffer is, however, of opinion that in any case 80 *mgrm.* SO_2 in 1 litre of wine or beer is, on prolonged use, calculated to produce disease in the intestinal channel, and he is probably in the right.

Kämmerer found (Report of the Seventh Congress of Bavarian Chemists at Speyer) in 1 litre, *mgrm.* SO_2:—

	Maximum.	Minimum.	Mean.
38 white wines . . .	210	3x	93
17 red wines . . .	83	12	36
9 sweet wines . . .	46	4	17

Herz found in 102 samples of beer (*Repertor. f. Anal. Chemie*, 1885, p. 59) from 0 to 89 *mgrm.* SO_2 per litre. To the last-mentioned beer calcium bisulphite had admittedly been added. Beer from the Court Brewery (Hofbräuhaus) at Munich, for which one-third of sulphured hops had been used, contained per litre from 1·8 to 2·6 *mgrm.* SO_2.

Kayser found in a sample of cider 730 *mgrm.* per litre.

Wines which have been heavily sulphured some time ago show a great increase of sulphates, so that a mean amount of 1·2 *grm.* potassium sulphate was found per litre (ranging upwards to 2·6 *grm.* instead of about 0·12 to 0·4 *grm.* per litre, as found in pure wine).

The maximum allowed for wine and beer in Austria is 8 *mgrm.* per litre, on the basis of the opinion of the medical faculty of March 19, 1887. The Bavarian representatives of applied chemistry had proposed at their congresses in 1885 10 *mgrm.* for wine, whilst Herz contended for 13·75 *mgrm.* per litre for beer.

The last numbers may be regarded as correct for beer, but for wine they are too rigid; at least 40 *mgrm.* per litre of wine might be permitted without injury. Still suspicion is never misplaced with respect to a poison of the rank of sulphurous acid. According to Nessler, 2·7 to 5·4 *mgrm.* SO_2 in a litre of wine are sufficient to prevent the ordinary diseases of wine, and 22 *mgrm.* are sufficient to suppress fermentation in must for fully twenty-eight days.

§ 234. *Salicylic Acid.*—Salicylic acid is recommended for the preservation of all possible organic substances; but, on account of its sparing solubility in water and its unpleasant taste, it is adapted merely for alcoholic liquids of powerful flavour. According to Prior, 0·05 *grm.* salicylic acid is sufficient to preserve beer, and for transmarine exportation he considers 0·2 *grm.* as admissible.

The toxicity of salicylic acid in very dilute solution is by no means considerable. Among others Kolbe took daily for a year 1 *grm.* salicylic acid in his various drinks without the

slightest injury. I allowed two labourers in Munich to take daily ½ *grm.* salicylic acid in ½ litre beer for seventy-five and ninety-one days respectively without detecting a trace of influence upon their health. Such a proportion can be recognised by the taste. Experiments on the influence of salicylic acid on the processes of digestion and the utilisation of food in healthy persons are wanting. In dogs, doses of sodium salicylate occasion an increased metabolism of albumen in the body (Wolfsohn, *Dissert.*, Königsberg, 1876, and C. Virchow, *Zeit. f. Physiolog. Chemie*, vi. 78).

Symptoms of cerebral poisoning have been observed on the ingestion of 6 to 12 *grm.* within a short time, but grave toxic phenomena have been even seen on the consumption of 4 *grm.* sodium salicylate. French authors especially call attention to the injurious action of salicylic acid in disease of the kidneys, and to its slow elimination in aged persons.

Concerning benzoic acid we have little information; it may for the present be adjudicated upon quite like salicylic acid, both from a qualitative and quantitative point of view.

Recently there have appeared recommendations of sodium fluoride as an "absolutely harmless" preservative agent for milk and butter. It is said to be very efficient (*Molk. Zeitung*, 1890, 39). Rabuteau was affected with salivation on taking 0·25 *grm.*; dogs vomit after taking 0·5 *grm.* internally. Larger doses produce cramps, paralysis, and death. See Tapeiner (*Archiv f. Experiment. Pathologie*, xxv. 203). The detection of sodium fluoride depends on the principle of liberating from it hydrogen fluoride, which etches glass. The dry substance is placed in a platinum capsule, set in a dish of warm water, and covered with concentrated sulphuric acid. Instead of the dry substance an aqueous extract evaporated to dryness may be used if more convenient. The capsule is covered with a watch-glass coated with wax, in which a letter has been inscribed by means of a splinter of wood. After some time we cleanse the watch-glass, and observe whether the letter appears faintly etched on the glass. By breathing upon it we may often render it more distinct.

§ 235. From what has been said above it appears that:—

1. If fresh articles of food are very cautiously mixed with quantities of boric acid, sulphurous acid, or salicylic acid exactly sufficient for their preservation, no disturbances of health will probably ever occur in healthy persons on a single ingestion of the substance, or on their use for a brief period.

2. On prolonged use, even a careful employment of boric acid does not seem indifferent, and the behaviour of sulphurous acid will be similar. No disturbances have as yet been observed from slight doses of salicylic acid, although an effect upon the utilisation of foods is perfectly possible.

3. But if an attempt is made to preserve food, already in incipient decomposition, by larger doses of these preservative agents, and if the daily dose of these substances is increased in consequence of the general and bolder use of preservative agents, there is for all three substances the decided possibility of an injury to health.

4. Most especially will this happen in case of children, sick persons, women during pregnancy or confinement, and aged persons.

If, therefore, we are not yet able to fix any definite minimum dose and minimum time of action at which the several preservative agents act harmfully, it must be demanded:—

1. That the kind and the quantity of the preservative agent added must be stated on the label, in default of which the food is to be at once objected to.

2. Milk, and all products especially recommended as nourishment for children, must not contain any preservative addition whatever. For milk, ebullition is the right preservative agent.

3. Additions which exceed the minimum quantity of the agent required for the preservation of fresh food must be condemned.

4. That no preservative agent should be sold as a secret nostrum.

5. That no new preservative agent shall be introduced into the market until unobjectionable experiments have

proved its innocuous character as compared with the substances already described.

Even if preservative agents are relatively harmless, when we decide upon them another consideration must be weighed. They facilitate an uncleanly, slovenly treatment of food; they render it possible to preserve articles in incipient decomposition for some time with the appearance of freshness, &c., and thus favour a series of frauds and tricks of trade, and in their train a number of menaces to our health. If we disregard very exceptional cases, chemical preservatives as an *addition* to foods and articles of consumption are superfluous. Their use in purifying vessels is somewhat different.

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